

PYTHIUM ROOT DIEBACK  
OF MUCK-GROWN CARROTS

By

RONALD J. HOWARD

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## INTRODUCTION

Carrots (Daucus carota L. var sativa DC.) are an important vegetable crop in the United States with an annual value exceeding \$100 million (7). Wisconsin ranks second nationally in production of processing carrots and a yearly harvest of approximately 4000 acres worth \$4-6 million makes this crop an important segment of the state's vegetable industry.

Commercial carrot production in Wisconsin is centered on organic (muck) soils. The limited availability of muckland and high costs of production have necessitated intensive carrot cropping on many muck soils. In a number of instances, pathogens have built up on these continuously cropped soils to the point where they have limited production. Among the soil pathogens of carrots, a root dieback has been observed with increasing frequency over the past several years.

Typical symptoms are a discoloration and necrosis of young lateral and taproots resulting in a stunting and forking of the carrots. A similar condition has been reported on carrots grown on organic soils in other locations (1,4,6), and recent studies have confirmed Pythium to be the causal agent, hence, the disease has been named Pythium root dieback (PRD).



At the beginning of this study, the etiology of PRD was not well defined (2,5,8). Because of widespread reports of similar root diseases on carrots and the consistent association of Pythium with them, we undertook a survey of Pythium species found in soils of several major carrot growing areas in the U.S. and Canada. Comparisons of the pathogenicity of these species and a study of their inoculum potential provided a means for assessing their involvement in PRD. In the course of this work, it became necessary to investigate the influence of soil type, moisture and depth, and cropping practices on populations of pathogenic and non-pathogenic species of Pythium.

Although reports of PRD were widespread, little documentation of incidence and yield losses existed, and in order to determine the importance of PRD relative to other root disorders, surveys and disease loss estimates were conducted over four growing seasons in Wisconsin.

Because of the concern over PRD expressed by carrot producers and processors, and the absence of information on its control, a number of control practices (chemical, cultural, crop rotation, disease resistance) were investigated. In order to gather basic information to assist in evaluating these practices, studies were made of the host range of several Pythium species and effects of soil moisture and varying extents of root injury on symptom development. In order to more clearly identify sources of resistance to

PRD for carrot breeding, a reliable disease screening method was developed.

This dissertation is presented in three parts and three appendices. Part I is a survey of species of Pythium encountered in carrot fields of North America and their relative virulence. In these studies, the relationship of Pythium inoculum potential to soil moisture and the incidence of PRD is investigated. In Part II, surveys of the incidence of PRD and other carrot root diseases, and of yield and monetary losses due to root diseases are determined for Wisconsin. Cultural aspects of PRD control are also examined. The development of reliable screening tests for Pythium root dieback and Rhizoctonia crown rot and cavity spot (3) disease resistance in carrots is described in Part III.

Appendix I reports the results of evaluating experimental chemicals for PRD control. Appendices II and III document optimal inoculation methods for detecting resistance in carrots to Pythium root dieback and Rhizoctonia crown rot and cavity spot. These appendices were written according to an established format to be used by plant breeders in screening breeding stocks for disease resistance.

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Incidence of Pythium Root Dieback of Carrots  
on Organic Soils

ABSTRACT

Ten Pythium species were identified among 518 isolates taken from diseased field carrots and seedlings grown in soil samples from 10 locations in the U.S. and Canada. P. irregulare and P. sulcatum, the most pathogenic species, and P. sylvaticum were the most frequently isolated. Pythium root dieback (PRD) of carrots occurred in most organic soils. With known inoculum levels of P. sulcatum and P. irregulare, statistically significant correlations existed for all combinations among 3 variables; seedling emergence, root dieback index (RDI) and Pythium populations (propagules/g), however, in field soils, significant correlations were obtained only for RDI x propagules/g and emergence x RDI. High soil moisture favored Pythium growth as indicated by a significant positive correlation between moisture and propagules/g. Comparable means and ranges existed for variables measured on samples from within and outside of Wisconsin. A smaller proportion of the total Pythium population in virgin organic soils was pathogenic. Pythium propagules/g decreased significantly with increasing soil depths. In organic soil, moisture retention was proportional to the percent organic matter.

## INTRODUCTION

Over the past several years wherever carrots have been intensively cropped on organic soils in North America, root disorders resulting in discoloration and necrosis of young lateral and taproots, and in subsequent stunting and forking of the carrots, have been observed. These diseases have been described variously as lateral root dieback (20), brown root (8) and rusty root (14), but are now collectively known as Pythium root dieback (PRD) (6,15,20). In a number of reports, Pythium species have been implicated as the causal agent or as associated with PRD (6,8,12,15,20). In addition, seedling damping off (6,8) and rubbery slate rot on stored carrots (13) have been attributed to Pythium spp.

Because of widespread reports of various root diseases resembling one another on carrots and due to the consistent association of one or more species of Pythium with PRD, a detailed survey of Pythium species found in the soils of several major carrot growing areas in the United States and Canada was undertaken. Comparisons of the pathogenicity of these species and a study of their inoculum potential provided a means for assessing their potential involvement in PRD.

Attempts were made to examine the relationships of soil type, moisture and depth, and cropping practices with populations of pathogenic and nonpathogenic species of Pythium. In the course of the survey, soil moisture was found to be a

major factor influencing populations, survival and disease development of Pythium, and it became important to examine the moisture characteristics of representative soils from which Pythium species were being isolated.

#### MATERIALS AND METHODS

Soil and carrot samples were obtained from fields in the major production areas of seven states in the U. S. and three Canadian provinces (Table 1). In some areas, samples were obtained from several fields and from fields planted to crops other than carrots. Soil samples were taken at 15-30 cm depths with a soil tube or hand trowel, composited in 1-liter plastic bags, and stored at 10 C. Cropping history, incidence and distribution of disease, and field location were recorded for each sample. When carrots with PRD were collected, care was taken to preserve the lateral root system.

Pythium species were isolated from diseased carrot roots and from seedlings grown in the soil samples. Two to 5-mm sized pieces of diseased roots were washed in running tap water for 2-3 hours, rinsed in sterile water, and transferred to agar plates. Larger pieces of diseased tissue were surface sterilized in sodium hypochlorite (1%) for 60 seconds, rinsed in sterile water and plated. Isolations were made on both water agar and cornmeal agar (CMA) amended with antibiotics, e.g., Ocana and Tsao's medium (9) or CMA, pimaricin (50 µg/ml)

and sodium novobiocin (50  $\mu\text{g ml}$ ). Plates were incubated at 20-26 C in the dark for 24-48 hours. Transfers were made from the margins of colonies onto CMA slants in test tubes, and after the isolates had grown they were stored at 10 C. Isolates were transferred to fresh CMA and potato dextrose agar plates for identification. Cultures of Pythium and Phytophthora were identified to species according to the keys of Waterhouse (16,17) and by reference to original species descriptions (7,12,18,19). Isolates of Pythium sylvaticum were verified by the induction of oospores using mating testors (4) on Schmitthenner's agar (17).

Pythium species were assessed for virulence on cv. Waltham Hi Color seedlings. Two isolates of each species except for P. afertile and P. splendens, were grown in a cornmeal-sand-muck mixture (CSM). Cornmeal-sand medium (CS) was prepared (11), inoculated with agar plugs colonized with Pythium, and after 2 weeks incubation at 20-24 C, cultures were shaken into plastic bags, mixed with steamed muck at a ratio of 1:4 (v/v) and grown for 1 week with daily mixing. Pythium populations were quantified by dilution plating as described below and diluted with steamed muck to a concentration of 1000 propagules/g.

Percent seedling emergence and root dieback indices (RDI) were determined for each Pythium spp. (Table 1) as described below. Root dieback index and emergence values of single pathogenic isolates of P. irregulare (FLOR 3) and P. sulcatum (BC 13) were compared at five population levels (Table 5)

replicated four times using the method above, and statistical correlations were determined among propagules/g, RDI and percent seedling emergence.

Soil samples were examined in five tests: 1) dilution plating for Pythium populations, 2) percent emergence of carrot seedlings, 3) percent soil moisture by weight, 4) percent die-back of seedling carrot roots (RDI), and 5) a root knot nematode index. Prior to testing, samples were passed through a 5-mesh screen. Those too wet to sieve were air-dried until they could be screened.

Total Pythium populations were determined by dilution plating on Ocana and Tsao's medium (9). To prepare soil: agar dilutions of 1:100, 1:50 and 1:25, subsamples of 2, 4 and 8-g of soil, respectively, were suspended with the aid of a magnetic stirrer in 200 ml aliquots of .25% water agar amended with 45,000 I.U. Penicillin G/liter. Ten g subsamples were oven dried at 108 C for 24 hours, cooled and reweighed to obtain an oven dry weight (ODW). One-ml aliquots of soil-agar suspension were removed while mixing and seeded on each of three plates of selective medium. The suspension was spread evenly over the agar surface with a bent glass rod and seeded plates were stored at 20 C in the dark for 24 hours before washing the suspension from the plate surface. Colonies were counted under a dissecting microscope and the number of Pythium propagules/g ODW soil was calculated.



Soils were assayed for PRD by measuring the percent emergence and root dieback of seedlings. Seedlings were grown at 20 C with 19,000 lux of mixed cool white fluorescent and incandescent illumination on a 12 hour photoperiod. Soil moisture was maintained at  $-.02$  to  $-.04$  bars matric potential at the porous ceramic plates of moisture tension columns (10).

Columns were filled with a soil sample to 8-cm depth and packed with  $.2\text{-kg/cm}^2$  of pressure. Fifty carrot seeds (cv. Waltham Hi Color) of predetermined germination percentage were sown, covered with 100-cc of soil and packed. Air was purged from the chamber beneath the porous plate and the soil saturated by raising the reservoirs above the columns and slowly adding water to the top of the soil. After saturation, the water reservoirs were lowered to a 20-cm position below the columns. Deionized water was used throughout. Ten days after seeding, emerged seedlings were counted and thinned to 10/column. Twenty-one days after seeding, the columns were immersed in water for 12 hours and the softened root mass and soil were washed carefully onto a 5-mesh screen. Individual root systems were teased apart under water and the percent root dieback was determined with the aid of a pictorial key (Fig. 1). The key was developed by tracing pressed specimens of cv. Waltham Hi Color seedlings grown in steamed muck in the moisture tension cylinders. Thickened lines were inked in on the original tracings to depict 25, 50, 75 and 100%

root dieback at various ages. The mean percent root dieback of plants in a sample was determined and referred to as the root dieback index (RDI).

Because root knot nematodes can cause taproot forking, soils were assayed for nematodes by growing a tomato, Lycopersicon esculentum Mill. (cv. Heinz 1370), in each sample. Eight to 10 weeks after transplanting 2-3 week old seedlings into the soil samples, roots were washed and examined for galls. Carrots grown in moisture tension columns were also observed for galls.

The vertical distribution of Pythium species in six Wisconsin muck soils was studied by sampling with a 5-cm diameter soil auger at 15-cm intervals to 120-cm depth. Pythium populations were determined by dilution plating and isolations of Pythium spp. were attempted from RDI seedlings.

In order to determine a suitable means for the long term storage of Pythium spp. collected during surveys, isolates were grown on different media in 15 x 45 mm screw cap vials held at 10 C in the dark. Isolates were stored on week-old colonized 1-cm sections of grass blades in sterile distilled water, in colonized 0.5-cm<sup>2</sup> CMA plugs in sterile distilled water, in steamed muck soil, and on CMA slants. After 16 months of storage, transfers were made to CMA and growth noted following 2-3 days at 20-24 C.

Water contents at ten matric potentials were determined for nine organic soil samples of varying texture and origin from Wisconsin and Quebec. Deionized water was added to

500-cc aliquots of soil and the mixture was stirred to a paste with a "Mixmaster." Matric potentials from  $-.02$  to  $-.08$  bars were obtained with a modified Haine's apparatus (5) while remaining potentials were obtained using pressure plate extractors (Soil Moisture Equip. Co., Santa Barbara, Calif.).

Percent organic matter was determined by incineration of weighed quantities of oven-dried soil in crucibles at  $580\text{ C}$  for 30 minutes in a muffle furnace and determination of the weight lost (1).

#### RESULTS

Ten species of Pythium were identified among 518 isolates taken from diseased field carrots and from seedlings grown in soil samples collected from seven states of the U.S. and three Canadian provinces (Table 1). P. irregulare, P. sulcatum and P. sylvaticum were the most frequently isolated and in inoculation tests with each species, P. irregulare and P. sulcatum were the most pathogenic. The greatest number of species were found in Wisconsin, perhaps, due to the fact that more Wisconsin samples were examined. No pathogenic Pythium species were isolated from plants grown in a mineral soil sample from Texas.

Pythium species were found in all soil samples from 67 fields (Tables 2, 3). RDI values indicated that pathogenic

Table 1. Classification, distribution and virulence of *Pythium* species from diseased roots of carrots grown in soil samples from nine locations in the U.S. and Canada.

Pythium isolate	Distribution and number of isolates									Virulence <sup>a</sup>		
	B.C.	Fla.	Mich.	Minn.	N.Y.	N.C.	Ont.	Que.	Wis.	Species total	Seedling emergence (%)	RDI
<u>P. afertile</u> Kanousa & Humphrey	0	0	0	0	0	0	0	0	1	1	28	0
<u>P. coloratum</u> Vaartaja	3	6	0	6	0	0	0	0	7	22	34	3
<u>P. irregulare</u> Buisman	16	17	0	0	1	3	7	5	81	130	4	86
<u>P. mamillatum</u> Meurs	0	0	0	0	0	0	1	0	4	5	50	5
<u>P. paroecandrum</u> Drechsler	6	2	0	0	0	0	0	0	11	19	6	39
<u>P. periiilum</u> Drechsler	0	2	0	1	0	0	0	0	0	3	42	8
<u>P. spinosum</u> Sawada	0	1	0	0	0	4	0	0	0	5	12	36
<u>P. splendens</u> Braun	0	0	0	0	0	0	0	0	1	1	58	10

(continued)

Table 1 (continued)

Pythium isolate	Distribution and number of isolates										Virulence <sup>a</sup>	
	B.C.	Fla.	Mich.	Minn.	N.Y.	N.C.	Ont.	Que.	Wis.	Species total	Seedling emergence (%)	RDI
<u>P. sulcatum</u> Pratt & Mitchell	3	11	0	0	1	1	0	28	77	121	20	60
<u>P. sylvaticum</u> Campbell & Hendrix	3	2	7	7	0	0	3	12	118	152	28	2
<u>P. ultimum</u> Trow	6	0	7	0	0	0	0	0	0	13	46	5
<u>P. vexans</u> deBary	0	1	0	0	0	0	0	1	2	4	72	0
Unclassified	4	6	3	1	0	0	2	4	22	42		
Total isolates	41	48	17	15	2	8	13	50	324	518		

<sup>a</sup>Virulence: 1) % seedling emergence from 50 carrot seeds, and 2) root dieback index (RDI) = area of carrot roots with dieback; mean of 2 isolates x 4 replicates. P. afertile and P. splendens had 4 measurements/mean.

<sup>b</sup>Two isolates of Phytophthora cactorum Lebert & Cohn were recovered from diseased roots of field-grown carrots; emergence = 75%, RDI = 7.

species of Pythium occurred in most organic soils. Root knot nematodes were detected infrequently, but where they occurred, damage was sometimes serious.

Wide ranges in the variables determined for each soil sample; Pythium propagules/g, percent seedling emergence, RDI and percent soil moisture, necessitated statistical evaluation of their interrelationships (Table 4). For both Wisconsin samples and those from elsewhere, highly significant positive correlations ( $P = .01$ ) existed between soil moisture and Pythium propagules/g soil. For samples from outside of Wisconsin the positive correlation between RDI and propagules/g was very highly significant ( $P = .001$ ). Wisconsin samples had a significant negative correlation ( $P = .05$ ) between seedling emergence and RDI.

When increasing populations of P. sulcatum and P. irregulare were added to steamed soil in moisture tension cylinders, seedling emergence decreased whereas RDI increased (Table 5). Significant correlations existed among seedling emergence, RDI and propagules/g. P. irregulare produced significantly more damping off than P. sulcatum.

Mean soil moisture values were lower for out-of-state than for Wisconsin fields, but other variable means were similar (Table 6). Comparisons between virgin and cultivated organic soils in Wisconsin revealed similar Pythium populations and percent seedling emergence means, but considerably higher RDI values occurred for the cultivated soils. Cultivated

Table 4. Correlation coefficients<sup>a</sup> among variables measured on soils from within and outside of Wisconsin.

Location <sup>c</sup>	Variables <sup>b</sup>			
	Seedling emergence (%)	RDI	Pythium (propagules/g)	Soil moisture (%)
Wisconsin:				
Seedling emergence (%)	1.00	-.22*	.10 <sup>ns</sup>	.05 <sup>ns</sup>
RDI		1.00	.06 <sup>ns</sup>	.17 <sup>ns</sup>
Pythium (propagules/g)			1.00	.25**
Soil moisture (%)				1.00
Out-of-Wisconsin:				
Seedling emergence (%)	1.00	.12 <sup>ns</sup>	-.02 <sup>ns</sup>	-.02 <sup>ns</sup>
RDI		1.00	.40***	.15 <sup>ns</sup>
Pythium (propagules/g)			1.00	.35**
Soil moisture (%)				1.00

<sup>a</sup>ns = nonsignificant at  $P \leq .05$ ; \* = significant at  $P = .05$ ; \*\* = significant at  $P = .01$ ; \*\*\* = significant at  $P = .001$  of a t-test.

<sup>b</sup>Variables: 1) % seedling emergence of 50 carrot seeds, 2) root dieback index (RDI) = area of carrot roots with dieback, 3) % soil moisture oven dry weight (ODW), and 4) Pythium population (propagules/g ODW soil).

<sup>c</sup>Wisconsin = 71 samples; out-of-Wisconsin = 55 samples.

Table 5. A comparison of the virulence between single isolates of Pythium irregulare and P. sulcatum at five population levels on seedling carrots, and a correlation analysis between three virulence and treatment variables.

Treatment	Virulence <sup>a</sup>		
	Seedling emergence (%)	RDI	
<u>Pythium</u> population (propagules/g):			
0	74 A	0 C	
50	26 B	14 B	
500	28 B	40 B	
1000	36 B	40 B	
5000	0 C	100 A	
<u>Pythium</u> species:			
<u>P. irregulare</u>	11 B	41 <sup>b</sup>	
<u>P. sulcatum</u>	22 A	37	
Correlation coefficient analysis: <sup>c</sup>			
Virulence and treatment variables			
	Seedling emergence (%)	RDI	<u>Pythium</u> (propagules/g)
Seedling emergence (%)	1.00	-0.53***	-0.54***
RDI		1.00	0.77***
<u>Pythium</u> (propagules/g)			1.00

<sup>a</sup>Virulence: 1) % seedling emergence from 50 carrot seeds, and 2) root dieback index = area of carrot roots with dieback; mean of 4 replicates. Common letters in columns denote values not significantly different at P = 0.05 of a LSD test.

<sup>b</sup>F-test value was nonsignificant at P = .05.

<sup>c</sup>\*\*\* = significant at P = .001 of a t-test.



Table 6. Number of samples, mean and range of assay results for variables for soils from carrot fields within and outside of Wisconsin.

Location	Variables <sup>a</sup>			
	Seedling emergence (%)	RDI	Pythium (propagules/g)	Soil moisture (%)
Wisconsin:				
Virgin soil - n	12	22	22	22
$\bar{x}$	36	6	2196	278
Range	22-84	0-30	0-9086	79-465
Field soil - n	76	80	80	80
$\bar{x}$	36	22	2188	175
Range	0-72	0-100	0-7091	49-275
Out-of-Wisconsin:				
n	84	88	59	59
$\bar{x}$	32	21	2260	123
Range	4-68	0-75	185-8858	15-221

<sup>a</sup>Variables: 1) % seedling emergence of 50 carrot seeds, 2) root dieback index (RDI) = area of carrot roots with dieback, 3) % soil moisture oven dry weight (ODW), and 4) Pythium population. (propagules/g ODW of soil).

muck soils in Wisconsin had a lower mean percent moisture than virgin soils.

Significant decreases in the number of Pythium propagules/g of soil occurred with increasing depth of soil (Table 7), and as expected, the percent soil moisture increased with increasing depth. However, no significant differences in RDI or percent seedling emergence occurred between soil samples at increasing depths. Four Pythium spp. were isolated from carrot seedling roots grown in samples taken below the 30-cm depth with the exception of the 45-60 cm zone. No isolations were made from plants grown in soil cores from 0-30 cm because all other surveys were made in this zone.

The superiority of CMA slants for the storage of Pythium cultures over the other substrates tested dictated its use in our studies.

Since moisture retention was proportional to percent organic matter in the nine samples examined, characteristic curves for only four representative soil types were plotted (Fig. 2). The mean soil moisture percentage for 80 Wisconsin soil samples was 175% (Table 6) approximating the theoretical value for field capacity (-0.33 bars).

#### DISCUSSION

The results of our study would indicate that various species of Pythium are endemic in a range of organic soils

Table 7. The incidence of Pythium species and Pythium root dieback (PRD) in samples of organic soil taken at eight depths.

Sampling depth (cm)	Variables <sup>a</sup>				Pythium species and no. of isolates			
	Seedling emergence (%)	RDI	Pythium (propagules/g)	Soil moisture (%)	<u>P. coloratum</u>	<u>P. irregulare</u>	<u>P. sulcatum</u>	<u>P. sylvaticum</u>
0-15	40	12	3186 A	154 C				
16-30	30	17	1194 BC	141 C				
31-45	28	20	2128 AB	218 BC	0	0	7	1
46-60	32	15	1994 AB	277 AB	0	0	0	0
61-75	32	18	1755 BC	296 AB	0	0	0	5
76-90	30	10	742 B	308 AB	0	0	0	2
91-105	44	12	680 C	307 AB	0	5	0	0
106-120	38	6	591 C	333 A	1	0	0	5

<sup>a</sup>Variables: 1) % seedling emergence of 50 carrot seeds, 2) root dieback index (RDI) = area of carrot roots with dieback, 3) % soil moisture oven dry weight (ODW), and 4) Pythium population (propagules/g ODW soil); mean of 6 fields. Columns minus letters had a nonsignificant F-test ( $P \leq .05$ ), otherwise common letters in columns denote means not significantly different at  $P = .05$  of an LSD test.

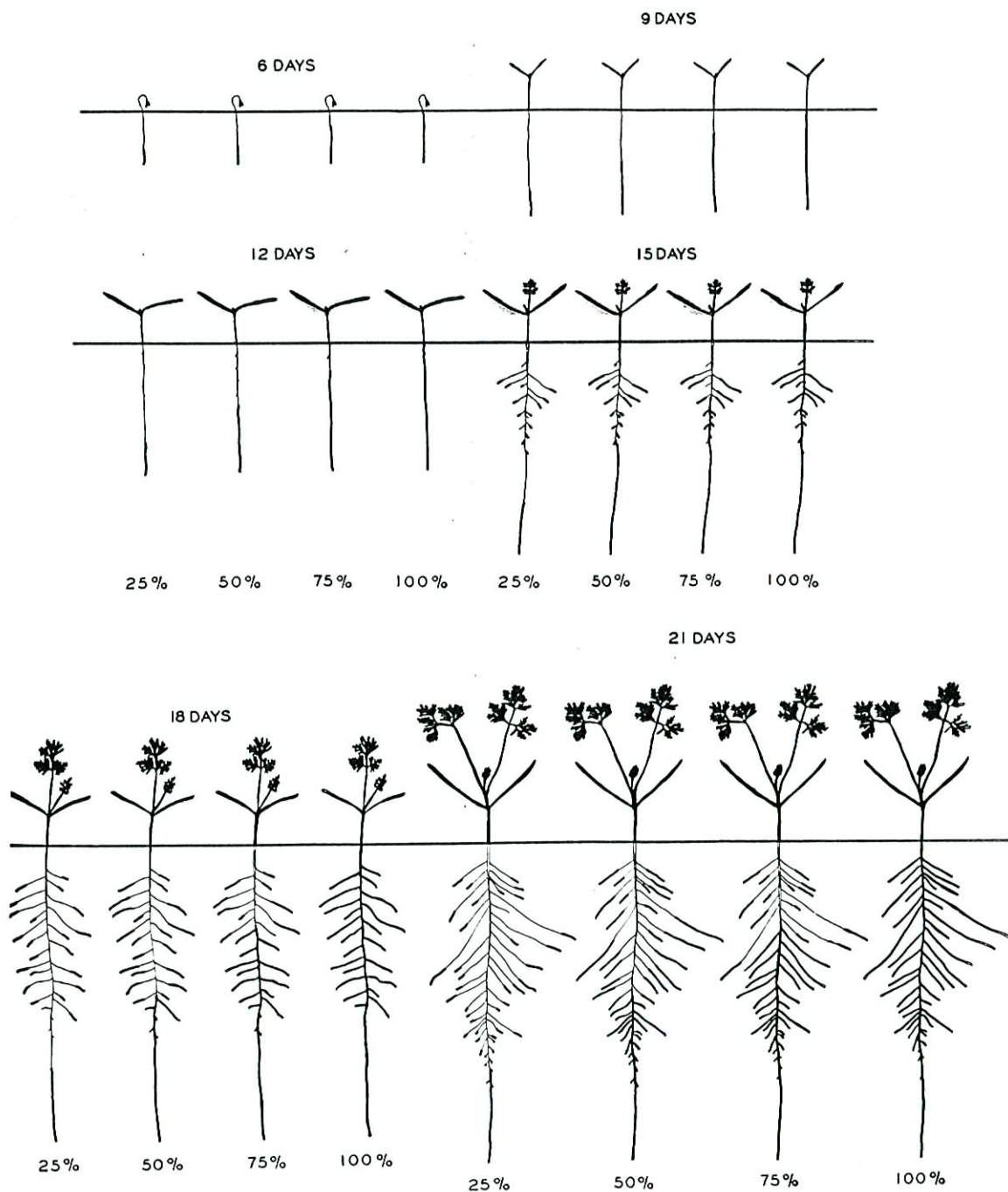


Figure 1. A pictorial key depicting four percentages of dieback on roots of cv. Waltham Hi Color seedling carrots of six ages.

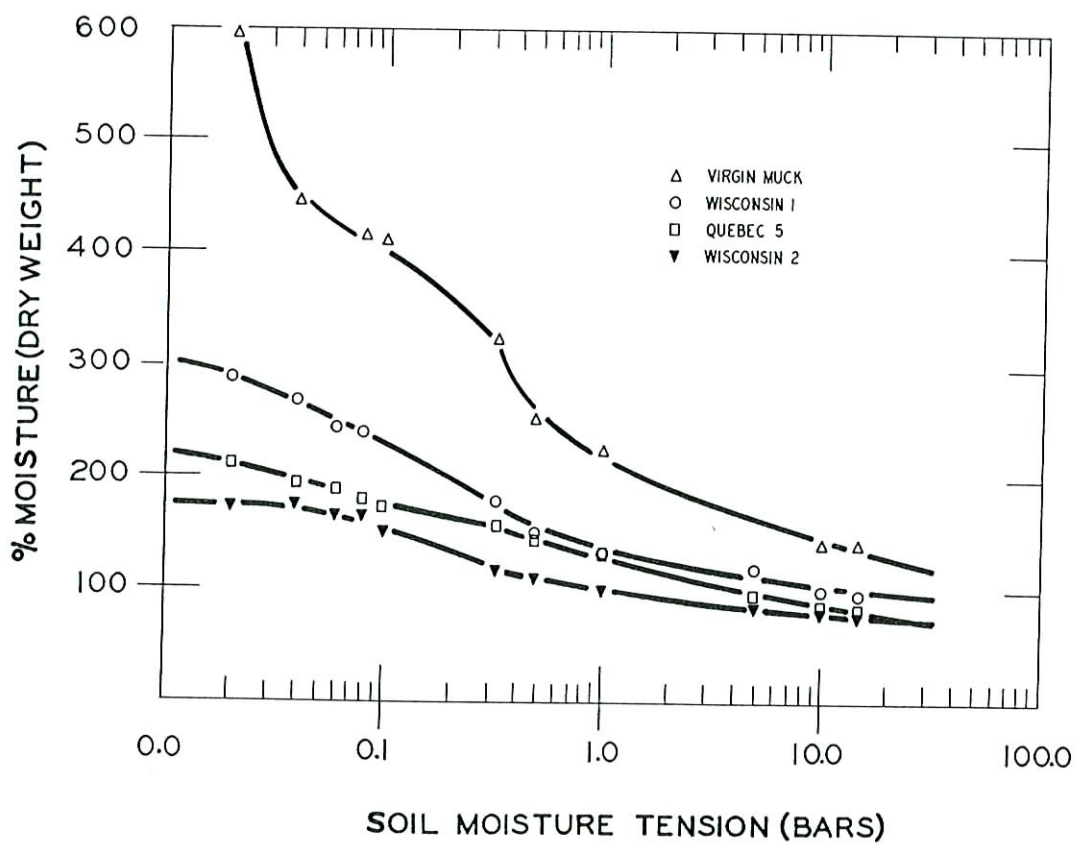


Figure 2. Moisture characteristic curves for organic soils from Wisconsin and Quebec. Points on curves = mean of 2 measurements. Organic matter contents by weight: Quebec 5 = 57%, Wisconsin 1 = 66%, Wisconsin 2 = 46% and Virgin muck = 70%.

in North America and that in a number of these soils where Pythium root dieback (PRD) has been reported one or more pathogenic species may be implicated in the disease. The widespread occurrence of the highly pathogenic P. irregulare and P. sulcatum suggests that these species may be major contributors to PRD in a number of locations (Table 1). The less frequent appearance of other less pathogenic species in scattered locations indicates that these other species may be important either alone or in conjunction with the more ubiquitous pathogenic species in causing disease. Our results would agree with reports (6,8,12,15,20) that various species of Pythium are associated with PRD. Pythium root dieback may therefore be considered to describe a complex of symptoms on carrot roots which may be brought about by one or more pathogenic Pythium spp. acting either singly or together. The species contributing to PRD may vary from one location to another and conceivably from one diseased plant to another within a single carrot field. Whether a number of other fungi commonly isolated from roots along with Pythium species are indirectly involved in PRD has not been fully studied (14).

Although considerable differences in pathogenicity occurred among the various Pythium spp. in Table 1, our limited work (2 isolates/species) does not rule out the possible existence of virulent and avirulent isolates of various species.

The failure to find consistent significant correlations between Pythium propagules/g, RDI and seedling emergence in the data from field samples (Table 4) was in contrast to data obtained from experiments using controlled inoculum levels of highly pathogenic species (Table 5). Nonsignificant correlations among seedling emergence, RDI and propagules/g in field soils (Table 4) may be influenced by the proportion and relative virulence of pathogenic Pythium species occurring naturally. Whereas, the significant correlations of increasing populations of P. sulcatum and P. irregulare with decreasing emergence and increasing RDI (Table 5) may be attributed to the increasing inoculum potential. Significantly decreased seedling emergence caused by P. irregulare may be due to its more rapid growth rate when compared with the equally pathogenic P. sulcatum (12). Overall similarity of mean values for seedling emergence, RDI and propagules/g among soils sampled throughout this survey may well reflect limitations on the disease potential brought about by the uniform conditions of the testing methods (Table 6). Thus, the low percent seedling emergence observed overall (Table 6) was due to substantial damping off which resulted when favorable conditions for PRD were maintained in the soil moisture tension columns.

The highly significant correlations between soil moisture levels and Pythium populations in samples from all areas confirms the fact that high soil moisture favors Pythium growth (2,3,5,20). Since several Pythium spp. are known to

be active at matric potentials well below field capacity (-0.33 bars) (2,5), moisture levels commonly found in Wisconsin organic soils (175%-Table 6) are unlikely to limit Pythium growth.

Although Pythium populations in virgin soils matched those for field soils, their lower RDI values indicated that a smaller proportion of the total was pathogenic. Evidence that a rapid buildup of pathogenic Pythium populations can occur was seen in a field of recently broken virgin soil where serious PRD resulted after only 2 years of carrot cropping. The higher percent moisture levels (Table 6) and moisture holding capacities of virgin soils over cultivated soils (Fig. 2) may explain why Pythium populations build up so rapidly in newly opened muck.

The decline in Pythium populations with increasing soil depth and soil moisture (Table 7) would appear to contradict the high correlation between soil moisture and Pythium populations. However, at increasing soil depths, oxygen may become limiting as moisture levels increase.

Although there were too few soil samples to determine whether statistically significant trends existed for percent emergence, propagules/g and RDI among different cropping regimes and between seasons (Table 2), previous studies have shown that such parameters can affect the incidence of PRD (8,14,20). Pythium populations and RDI values appeared to be lower under rotations including potatoes and peppermint than with carrots alone (Table 2).



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Methods for Detecting Resistance to  
Pythium and Rhizoctonia Diseases  
in Seedling Carrots

ABSTRACT

Two methods of screening carrots for resistance to Pythium root dieback (PRD) and Rhizoctonia crown rot and cavity spot (CRCS) under controlled temperatures (24°C) and soil matric potentials (-100 ± 50 mb) are described. Carrots to be screened for PRD resistance are sown into steamed muck overlying a layer of Pythium sulcatum cornmeal-sand-muck inoculum (1000 propagules/g). To identify plants resistant to CRCS, carrots are seeded in steamed muck, grown to 4 weeks of age and inoculated with Rhizoctonia solani-infested corn kernels. Carrots are harvested at 16-20 weeks of age in both tests and assessed for resistance based on numbers of normal and abnormal roots produced.

INTRODUCTION

Crown rot and cavity spot (CRCS) caused by Rhizoctonia solani Kühn, and root dieback (PRD) incited by various species of Pythium are important diseases of carrots grown on organic

soils in Wisconsin and other areas of North America (1,2,5,9, 10,11). A number of cultural and chemical controls for CRCS and PRD have been evaluated (2,4,10,11) and found to be of potential value, however, these practices would be more effective if combined with resistant cultivars. At this time, reports of resistance to CRCS and PRD in carrots have been based upon empirical observations in the field under conditions of varying inoculum and environment and all commercial cultivars are more or less susceptible. In order to more clearly identify sources of resistance for PRD and CRCS for carrot breeding, reliable screening methods were needed.

By optimizing pathogen inoculum and host uniformity along with environmental conditions under which disease develops, variations in susceptibility within a carrot population should be limited to the expression of genetic differences. Thus by examining a range of environmental conditions, stages of host growth, and methods of inoculum preparation, we have established conditions suitable for the selection of genetic resistance to PRD and CRCS in carrots.

#### MATERIALS AND METHODS

Since CRCS and PRD develop on carrot roots, control of soil type, moisture and temperature is essential. A uniform source of organic (muck) soil containing 60-65% organic matter with a known moisture characteristic curve (2) was

used throughout these experiments. Soil temperatures were controlled using Wisconsin soil temperature tanks (3) and moisture was regulated with LARK manometers (Soilmoisture Equip. Corp., Santa Barbara, Calif.) (Fig. 1).

Mercury manometers connected through 1 m lengths of Nulo-Seal plastic tubing (Imperial Eastman, Chicago, Ill.) (2 mm I.D.) were cemented into 10 cm porous ceramic tubes (Selas Flotronics, Drescher, Pa.) with a 0.8  $\mu$  pore size and .83 kg/cm<sup>2</sup> (12 p.s.i.) bubbling pressure (3.2 mm I.D.). Thirty-cm lengths of Nulo-Seal tubing with airtight, screw cap brass Swagelok fittings (Crawford Fitting Corp., Cleveland, Ohio) were cemented into the other end of the ceramics. Freshly boiled deionized water was introduced into manometer lines with the aid of a hypodermic syringe through the opened fitting, and was flushed through lines weekly. After use, ceramics and tubes were disinfected in formalin (10%) for 4 hours.

In experiments with Pythium root dieback, the highly virulent P. sulcatum Pratt & Mitchell (9) was used. Populations (propagules/g oven dry soil) were readily quantified by growing the fungus initially on a cornmeal-sand mixture (CS) which could be easily mixed with soil. P. sulcatum was grown on CS (8) inoculated with agar plugs of 5 isolates (1 isolate/flask) and after 2 weeks of incubation at 20-24°C, the 5 cultures were shaken together into a plastic bag, mixed with steamed muck at a ratio of 1:4 (v/v) and grown for 1 week with

daily mixing. Propagules/g in the cornmeal-sand-muck mixture (CSM) were determined by dilution plating on Ocana and Tsao's selective medium (7). The CSM was diluted with steamed muck to 1000 propagules/g. This population level was previously shown to cause severe root dieback and damping off on carrots (2).

To determine whether CSM inoculum could be efficiently utilized after storage at 10°C, subsamples of inoculum were sown with 50 seeds every 30 days for 6 months and the severity of PRD observed.

Since seedling emergence was seriously reduced by seeding directly into CSM inoculum (1000 propagules/g) a method of separating the inoculum a sufficient distance from the seeds to permit uniform emergence was established empirically. Two-3 cm of CSM inoculum were layered in the bottom of a stainless steel pan and two ceramic tubes were embedded approximately 20 cm apart. The inoculum was covered with 7 cm of steamed muck. Carrots were seeded 1.5 cm deep, 1 cm apart with 4.5 cm between rows. At 4 weeks, seedlings were thinned to 3 cm apart in the row. Water was added to the soil surface to maintain a matric potential of  $-100 \pm 50$  mb, soil temperature was held at 24°C, and plants received a 12 hour photoperiod.

In studies of CRCS, a highly virulent isolate of Rhizoctonia solani, R-3 (6), was used. Autoclaved corn kernels (6) provided an easily prepared quantifiable inoculum. Kernels colonized for five lengths of time were prepared by

inoculating flasks of corn with plugs of 7 day old cultures of R. solani on cornmeal agar. Kernels were incubated at 20-24°C for 2 weeks with mixing every 2-3 days.

Carrots grown in muck soil under conditions of uniform moisture and temperature were exposed to various ages of inoculum (Table 1). Pans were filled with 10 cm of steamed muck and manometer ceramic tubes were buried 7 cm below the surface 20 cm apart. Carrots were seeded in rows 4.5 cm apart and thinned to 1 cm between plants after 3 weeks. One week later, carrots were inoculated by burying a solid line of kernels 1 cm away from the crown and 1.5 cm deep on both sides of the row. Soil temperature, moisture and illumination were as for the Pythium method. Fourteen weeks after seeding, roots were harvested and recorded as normal (healthy) or abnormal (rotted, undersized and misshapen).

In order to determine the most suitable age to inoculate carrots with R. solani, carrots of various ages (Table 2) were inoculated as stated above.

The effect of soil moisture and temperature on PRD and CRCS development was examined by growing carrots under soil moistures held at various levels from 0 to -400 mb, and at various temperatures (Table 3). Plant growth and symptom development were recorded as a function of soil moisture and temperature.



## RESULTS

Symptom development for PRD and CRCS differed. For Pythium, damping off occurred soon after emergence and continued up to 4-6 weeks. Plants severely affected with PRD were stunted and slightly chlorotic. At harvest, affected roots had varying amounts of dieback on lateral and taproots and some taproots were misshapen (forked, stubbed) and undersized. Resistant roots were normal-shaped and uniform in size.

With Rhizoctonia, crown rotting was first observed about 2 weeks after inoculation and appeared as a collapse of the older leaves followed by wilting, chlorosis and death of tops. The greatest number of plants were affected by this phase of the disease and were dead by 4-5 weeks after inoculation. Thereafter, most plants died from cavity spot and subsequent rotting of the roots rather than of the crown. At harvest, roots were sorted into normal (healthy) and abnormal (rotted, undersized and misshapen) groups.

In evaluating the effect of inoculum age on severity of PRD, no differences could be seen among plants grown in samples up to 6 months old. For CRCS, there were no significant differences in numbers of normal or abnormal roots for 2, 4, 8 and 12 week-old inoculum, or between cultivars inoculated with inoculum of any age (Table 1).

Table 1. The effect of inoculum age and carrot cultivar on Rhizoctonia crown rot and cavity spot.

Treatment	Roots/row <sup>a</sup>	
	Normal	Abnormal
<u>Inoculum age (weeks)</u>		
2	0.4 B	0.0
4	1.1 AB	0.4
8	1.3 AB	0.8
12	0.8 B	1.4
16	2.4 A	0.5
<u>Cultivars</u>		
Royal Chantenay	.9	.8
Scarlet Nantes	1.5	.5

<sup>a</sup>Out of 25 plants/row; mean of 4 replicates. Normal = healthy, abnormal = rotted, undersized or misshapen. Common letters in columns denote means not significantly different at  $P = .05$  of a LSD test. Columns minus letters had a non-significant F-value at  $P \leq .05$ .

When carrots of various ages were tested for susceptibility to CRCS, those inoculated at 2, 3, 4 and 6 weeks of age were not significantly different for numbers of normal or abnormal roots (Table 2). The greatest number of survivors (normal and abnormal) occurred in the groups inoculated at seeding (0 weeks) and 8 weeks. There were no significant differences between cultivars.

Soil moisture could be reliably maintained at potentials ranging from 0 to -400 mb with the aid of manometers. At potentials less than about -250 mb the soil surface frequently dried out and carrot germination and Rhizoctonia inoculum survival were reduced whereas soil held at 0 mb was too wet for good carrot growth. Plant and disease development appeared optimal at potentials near -100 mb.

No significant differences in numbers of normal and abnormal roots of 2 cultivars could be detected between 20, 24 and 28°C (Table 3) for both CRCS and PRD, however, at temperatures of 32 and 36°C carrots grew poorly.

#### DISCUSSION

By examining various factors involved in inoculum preparation, host production, inoculum presentation and symptom expression, regimes have been developed which provide reliable screening of carrot populations for resistance to Rhizoctonia solani and Pythium sulcatum. When uniform, viable

Table 2. The effect of carrot age and cultivar on Rhizoctonia crown rot and cavity spot.

Treatment	Roots/row <sup>a</sup>	
	Normal	Abnormal
<u>Carrot age (weeks)</u>		
0	7.6 A	2.4 B
2	2.0 B	0.5 C
3	0.6 B	0.0 C
4	0.4 B	0.0 C
6	0.5 B	0.4 C
8	1.1 B	4.5 A
<u>Cultivars</u>		
Royal Chantenay	2.2	1.6
Scarlet Nantes	1.9	1.0

<sup>a</sup>Out of 25 plants/row; mean of 4 replicates. Normal = healthy, abnormal = rotted, undersized or misshapen. Common letters in columns denote means not significantly different at P = 0.5 of an LSD test. Columns minus letters had a nonsignificant F-value at P  $\leq$  .05.

Table 3. The effect of soil temperature and carrot cultivar on Pythium root dieback and Rhizoctonia crown rot and cavity spot.

Treatments	Pythium		Rhizoctonia	
	Roots/row <sup>a</sup>		Roots/row <sup>a</sup>	
	Normal	Abnormal	Normal	Abnormal
<u>Temperature (°C)</u>				
20	3.8 A	5.1 AB	2.6 B	.3
24	3.8 A	3.6 B	2.9 B	.5
28	4.0 A	4.0 B	.8 B	.1
32	2.9 A	6.0 A	6.1 A	.3
36	.4 B	1.1 C	1.8 B	.3
<u>Cultivar</u>				
Royal Chantenay	2.7	4.8 A	2.6	.3
Scarlet Nantes	3.3	3.2 B	3.1	.3

<sup>a</sup>Out of 10 plants/row; mean of 4 replicates. Normal = healthy, abnormal = rotted, undersized or misshapen. Common letters in columns denote means not significantly different at P = .05 of a LSD test. Columns minus letters had a nonsignificant F-value ( $P < .05$ ).

inoculum was presented to plants of the proper age, a predictable pattern of symptom development occurred and results were reproducible from pan to pan, and test to test.

Although inoculum age appeared to have little effect on PRD or CRCS we normally prepared it fresh before each test to assure cultures are actively growing and free from contaminating bacteria.

When susceptibility to CRCS among various ages of carrots was studied, our results were in agreement with those of Mildenhall and Williams (6), and it was most convenient to thin carrots at 3 weeks of age and inoculate them a week later.

At potentials of -50 to -150 mb the soil was moist to within a few millimeters of the surface permitting uniform seed germination and colonization by Rhizoctonia. During bright days in the greenhouse it may be necessary to water pans lightly 3-4 times to maintain sufficient surface moisture for germination or to keep potentials between -50 and -150 mb in established stands. The addition of water applicators electronically regulated through manometer sensors should minimize soil moisture fluctuations.

For screening of carrots against PRD and CRCS, 24°C is both convenient and suitable. Although soil temperatures were carefully maintained in our studies, the effectiveness of the tests over a range of temperatures (20-28°C) suggests that screening will be effective under less closely regulated conditions of temperature.

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## INTRODUCTION TO APPENDICES I, II, AND III

An increased awareness by Wisconsin carrot growers of the seriousness of Pythium root dieback (PRD) became apparent during the course of our field surveys. Considerable interest was expressed in obtaining a chemical which might provide a readily adaptable and rapid solution to the PRD problem. A search of the literature and personal communications with researchers in other locations where PRD occurred revealed that commercial chemicals were largely ineffective for PRD control. During 1973-74, we evaluated the efficacy of four experimental products in a Wisconsin field with a history of severe PRD. This research is presented in Appendix I.

If PRD-resistant carrot cultivars were available, they could be integrated with other methods of control. Since all commercial cultivars are more or less susceptible to PRD, there is a need to more clearly identify sources of resistance for carrot breeding. To accomplish this, a reliable and reproducible disease screening method is needed which differentiates between resistant and susceptible individuals in a population.

The clear expression of resistance or susceptibility requires careful control of the host, the pathogen and the environment so that differences expressed among individuals

is primarily of a genetic basis. Appendices II and III document screening methods for Pythium root dieback and for Rhizoctonia crown rot and cavity spot. The methods are presented in a format standardized to conform with screening methods presented for various diseases on a number of other vegetable crops and designed to be of use to plant breeders.

APPENDIX I

Chemical Control of Pythium Root Dieback  
of Muck-Grown Carrots

Chemical Control of Pythium Root Dieback  
of Muck-Grown Carrots

INTRODUCTION

The effectiveness of several commercial chemicals for the control of Pythium root dieback (PRD) was studied in British Columbia (5), Ontario (4), and Florida (3), but none significantly reduced disease in field trials. The widespread interest by Wisconsin growers in a chemical control for PRD led us to evaluate four experimental products for field efficacy: 1) potassium (KN<sub>3</sub>), and 2) sodium azide (NaN<sub>3</sub>) (PPG Co., Pittsburgh, Pa.), both broad spectrum pesticides (1,2), 3) Dowco 269 [2-chloro-6-methoxy-4-(trichloromethyl)pyridine] (Dow Chemical Co., Midland, Mich.), a systemic compound reported to control Pythium and Phytophthora, and 4) PP 395 [4-(3-chlorophenylhydrazono)-3-methyl-5-isoxazolone] (ICI Co., Ltd., Goldsboro, N.C.), a broad spectrum fungicide with a high activity against Pythium.

METHODS AND MATERIALS

Potassium azide: 1973 studies. In order to test the effect of potassium azide, irrigation and cultivar (Table 1) on the incidence of PRD in muck soil, a split plot

trial with three replicates was established. Azide (10% a.i.) was broadcast at 60 kg/ha a.i. and incorporated 15-25 cm deep with a rototiller. Check plots were only rototilled. Composite soil samples were taken from all subplots before azide application and examined for percent seedling emergence, Pythium populations (propagules/g), root dieback index (RDI) and percent soil moisture (see Part I). To avoid phytotoxicity, subplots were seeded 2 weeks after treatment. Soil samples were taken from treated subplots and cultivars were planted in 7.5 m rows at 500 seeds/row.

A solid set sprinkler irrigation system was used to apply a total of 12, 24 and 36-cm of water to designated plots in equal weekly increments during the first 8 weeks of crop growth. Three-m of row per cultivar were harvested 14 weeks after seeding and soil samples were taken.

Potassium azide: 1974 studies. An attempt was made to determine the best time during the spring to apply potassium azide and plant a crop for maximum PRD control. We evaluated several dates of azide application and planting (Table 3). Azide was applied as in 1973 using a randomized complete block design with four replicates. Composite soil samples were taken before azide treatment and at seeding. Carrots were sown in 3.5-m rows at 200 seeds/row. Entire rows of each subplot were harvested 16 weeks after seeding and soil samples were again taken.

Sodium azide. The effect of applying different rates of sodium azide (Table 5) on PRD was examined using a randomized complete block design with four replicates. The sodium azide (8% a.i.) was applied and incorporated as for the potassium azide. Cultivars were sown in 3.5 m rows at 200 seeds/row. Entire rows of each subplot were harvested 13 weeks after seeding. Composite soil samples were taken before treatment, at seeding and at harvest.

Dowco 269-PP 395 seed treatment. An evaluation was made of the effectiveness of Dowco 269 (5% a.i.) and PP 395 (40% a.i.) as seed dressings for PRD control. A split plot design was used with four rates of chemical and four carrot cultivars (Table 7). Small seedlots were treated with slurry preparations of Dowco 269 and PP 395 while a control received distilled water. Germination of samples of 400 seeds was determined for all treatments. Seed was planted by hand in 1.8-m rows and entire rows of each subplot were harvested 16 weeks later.

Dowco 269-PP 395 soil furrow treatment. The ability of Dowco 269 and PP 395 to control PRD when applied as a furrow treatment was examined. A split plot design was used with four rates of chemical and four carrot cultivars (Table 9). Chemicals were applied in a 2.5 x 180-cm furrow using a constant 100-ml of solution. Seeds were planted 100/row by

hand and treatments were replicated four times. Entire rows of each subplot were harvested 14 weeks after seeding.

### RESULTS

Potassium azide: 1973 studies. When the effect of irrigation on PRD and azide activity were examined, no significant differences in yield occurred between irrigation levels for any of the yield variables (Table 1). Azide-treated subplots produced significantly greater total plant and root weights than the untreated subplots, but no differences occurred for other yield variables. Spartan Bonus consistently yielded highest for all variables, Waltham Hi Color and Spartan Fancy yielded poorly whereas Danvers 126, Royal Chantenay and Scarlet Nantes were intermediate. Spartan Bonus was not significantly different from Royal Chantenay and Scarlet Nantes in yield of normal roots.

Among the variables, propagules/g and percent soil moisture for the 12-cm level of irrigation were significantly greater than those for 24- and 36-cm (Table 2). Among treated subplots over the season, seedling emergence was significantly greater among samples collected at seeding time whereas propagules/g were significantly lower and RDI values were not significantly different. At harvest, emergence and propagules/g were not significantly different from seeding.

Potassium azide: 1974 studies. In examining the effect of potassium azide and cultivars on PRD, we found Danvers 126 significantly outyielded Waltham Hi Color for all yield variables except abnormal root number and weight (Table 3). Significant differences occurred between levels of the azide application-planting date treatment for each yield variable as revealed by an analysis of variance. To facilitate a comparison among azide treatment dates, the eight levels were divided into four groups: controls (1,2,3,4), May 9 (5,6,7), May 21 (8,9) and June 6 (10). When LSD comparisons of yield means were made among azide application dates, no single date had a significantly higher marketable root yield (numbers and weight of normal roots) than all other dates. In order to compare control vs chemical treatment levels, a second ordering was done according to seeding date: 1) 2 with 5; 2) 3 with 6 and 8; 3) 4 with 7, 9 and 10; and 4) 1 was unpaired. No chemical treatment level had significantly higher marketable root yields than the maximum yield for controls.

When soil taken from azide treatment-planting date plots was examined, significantly greater seedling emergence and lower soil moisture occurred in soil samples taken at harvest (Table 4). Control levels generally had higher emergence, propagules/g and soil moisture values than azide levels whereas there were few significant differences among levels for RDI.



Sodium azide. In evaluating the effect of azide rates on incidence of PRD, carrot yields for the 30 and 60 kg/ha rates were found not to be significantly different from one another or the control for any yield variables (Table 5). The 90 kg/ha level yielded significantly less than the 0, 30 and 60 kg/ha levels except for number and weight of abnormal roots for which there were no significant differences between all levels.

Scarlet Nantes produced significantly more normal and fewer abnormal roots than other cultivars (Table 5). Waltham Hi Color yielded poorly except for number and weight of abnormal roots while Danvers 126, Red Cored Chantenay and Spartan Fancy were intermediate.

An analysis of soil from plots treated with sodium azide revealed seedling emergence was significantly higher and soil moisture lower at harvest (Table 6). Propagules/g were significantly lower at seeding and harvest than pre-azide. Only 60 and 90 kg  $\text{NaN}_3$ /ha significantly reduced Pythium populations over the control whereas 30 kg/ha was not significantly different from other rates.

Dowco 269/PP 395 seed treatment. An examination of the effect on germination when carrot seed was treated with Dowco 269 and PP 395 showed seed treated at 5000 mg/kg had significantly lower germination than other rates (Table 7). Germination of Dowco 269 treated seeds was significantly higher than for PP 395.

Carrots treated with PP 395 had a significantly greater number and weight of plants, number of normal roots and total root weight than Dowco 269, but no significant differences occurred for other yield variables (Table 8). The 1000 mg/kg rate produced significantly greater total plant, abnormal root and total root weights than the control, but the 500 and 5000 mg/kg rates were not significantly different from the control for any yield variables except abnormal root weight.

Royal Chantenay consistently yielded highest except for abnormal roots for which Waltham Hi Color yielded highest (Table 8). Danvers 126 and Spartan Fancy generally yielded less than Royal Chantenay.

Dowco 269-PP 395 soil furrow treatment. A study of the efficacy of Dowco 269 and PP 395 as soil furrow treatments for PRD control revealed there were no significant differences between chemicals or rates for any yield variables. Royal Chantenay consistently yielded highest except for abnormal roots for which Waltham Hi Color yielded highest (Table 9). Danvers 126 and Spartan Fancy generally yielded less than Royal Chantenay.

#### DISCUSSION

Among the four experimental fungicides evaluated for their ability to control PRD, none were found to be totally

effective based on comparisons of several yield variables, especially marketable yields (weight and number of normal roots). Although the azide treatments often reduced Pythium propagules/g by as much as one-half pretreatment levels, fungus populations usually recovered and marketable yields were no greater than for controls. An observation (see Part I) that Pythium species occur below the plow layer in muck fields suggests that the fungus could recolonize azide-treated soil from below. Our negative results with azide for PRD control correspond to reports from Florida (3). Negative results with Dowco 269 and PP 395 may have been partially due to the inability to concentrate enough chemical near the seedling roots. As expected, despite the ineffectiveness of chemical treatments, the more tolerant cultivars, Scarlet Nantes and Royal Chantenay generally yielded more marketable roots than the PRD susceptible Waltham Hi Color.

Table 1. The effect of irrigation, potassium azide treatments and carrot cultivar on yield in 1973.

Treatment <sup>a</sup>	Yield variables <sup>b</sup>						
	Plant wt. (kg)	Plant No.	Normal root no.	Normal root wt. (kg)	Abnormal root no.	Abnormal root wt. (kg)	Total root wt. (kg)
Irrigation (cm):							
12	24.7	161.7	85.3	8.5	76.4	8.1	16.6
24	20.8	142.8	83.9	7.3	58.9	6.0	13.3
36	21.8	148.9	90.7	8.9	58.3	6.3	15.2
Chemicals:							
Azide	22.8 A	161.6	89.5	8.1	72.1	7.1	15.1 A
Control	18.8 B	140.7	83.7	7.2	56.9	5.6	12.8 B
Cultivar:							
Waltham Hi Color	16.1 DE	121.5 B	53.3 C	3.8 C	68.2 BC	6.2 B	9.9 C
Danvers 126	25.7 AB	157.8 B	77.3 BC	6.9 B	80.5 AB	8.7 A	15.6 B
Royal Chantenay	22.3 BC	151.5 B	105.5 AB	10.1 A	46.0 D	4.5 C	14.6 B
Spartan Bonus	28.2 A	211.5 A	120.8 A	10.9 A	90.7 A	8.6 A	19.4 A
Spartan Fancy	13.3 E	103.2 C	57.8 C	5.1 BC	45.3 D	4.7 BC	9.8 C
Scarlet Nantes	19.2 CD	161.3 B	104.8 AB	9.2 A	56.5 CD	5.3 BC	14.5 B

<sup>a</sup>Irrigation was applied in equal weekly increments during the first 8 weeks of crop growth. Azide was broadcast at 60 kg/ha a.i. and rototilled in. Three replicates/treatment.

<sup>b</sup>Each variable represents mean yield/9-m row. Plant wt. = foliage + roots, abnormal roots = forked, stubbed, undersized or rotted. Common letters in columns denote means not significantly different at  $P = .05$  of a LSD test. Columns minus letters had a non significant F-value at  $P < .05$ .

Table 2. The effect of irrigation and sampling date on variables related to Pythium root dieback potential.

Treatment <sup>a</sup>	Variables <sup>b</sup>			
	Seedling emergence (%)	RDI	<u>Pythium</u> (propagules/g)	Soil moisture (%)
Irrigation (cm):				
12	48	27	3858 A	220 A
24	41	18	2272 B	153 B
36	48	25	2386 B	147 B
Sampling date:				
pre-azide	42 B	24	2996 A	183
seeding	56 A	30	1632 B	159
harvest	40 B	17	3888 A	179

<sup>a</sup>Irrigation was applied in equal weekly increments during the first 8 weeks of crop growth. Azide was broadcast at 60 kg/ha a.i. and rototilled in. Treatments were replicated 3 times.

<sup>b</sup>Variables: 1) % seedling emergence of 50 carrot seeds, 2) root dieback index (RDI) = area of carrot roots with dieback, 3) soil moisture oven dry weight (ODW), and 4) Pythium population (propagules/g ODW). Common letters in columns denote means not significantly different at  $P = .05$  of a LSD test. Columns minus letters had a nonsignificant F-value at  $P \leq .05$ .

Table 3. The effect of carrot cultivar and potassium azide application-planting date on yield in 1974.

Treatment <sup>a</sup>	Yield variables <sup>b</sup>					
	Plant wt. (kg)	Plant no.	Normal root no.	Normal root wt. (kg)	Abnormal root no.	Total root wt. (kg)
Cultivar:						
Waltham Hi Color	3.7 A	26.3 A	16.9 A	1.5 A	9.2	2.2 A
Danvers 126	6.0 B	37.8 B	28.8 B	2.9 B	8.8	3.7 B
Azide-planting date:						
1	6.0 AB	38.5 BC	31.9 B	3.2 AB	6.3 BC	3.9 AB
2	7.8 A	64.5 A	46.4 A	3.6 A	16.9 A	4.8 A
3	5.1 BC	31.0 BCD	21.3 BC	2.1 C	9.8 B	2.9 BC
4	2.1 D	10.4 E	7.8 D	1.1 D	2.6 C	1.4 D
5	5.7 ABC	41.0 B	20.8 C	1.7 C	19.0 A	3.1 BC
6	5.4 BC	33.6 BCD	25.5 BC	2.2 C	8.3 BC	3.1 BC
7	4.8 C	28.4 BCD	22.3 BC	2.5 BC	6.1 BC	3.1 BC
8	3.9 CD	25.8 CD	17.6 CD	1.6 CD	8.1 BC	2.4 CD
9	4.4 C	26.3 BCD	18.8 C	2.1 C	7.5 BC	2.9 BC
10	3.6 CD	21.1 DE	16.0 CD	1.6 CD	5.1 BC	2.1 CD

<sup>a</sup>Azide application - planting date:

1 = no azide, seeded May 9      5 = Azide May 9, seeded May 23      9 = Azide May 21, seeded June 20  
 2 = no azide, seeded May 23      6 = Azide May 9, seeded June 6  
 3 = no azide, seeded June 6      7 = Azide May 9, seeded June 20      10 = Azide June 6, seeded June 20  
 4 = no azide, seeded June 20      8 = Azide May 21, seeded June 6  
 Azide broadcast at 70 kg/ha and rototilled in. Four replicates/treatment.

<sup>b</sup>Each variable represents mean yield/3.5-m row. Plant wt. = foliage + roots, abnormal roots = forked, stubbed, undersized or rotted. Common letters in columns denote means not significantly different at  $P = .05$  of a LSD test. Columns minus letters had a nonsignificant F-value at  $P < .05$ .

Table 4. The effect of sampling date and potassium azide application-seeding date on variables related to Pythium root dieback potential.

Treatment <sup>a</sup>	Variables <sup>b</sup>			
	Seedling emergence (%)	RDI	Pythium (propagules/g)	Soil moisture (%)
Date of sampling:				
pre-azide	28 B	28	1763	204 A
seeding	30 B	21	1649	195 A
harvest	42 A	25	1476	164 B
Azide-planting date:				
1	44 A	21 B	2389 A	210 AB
2	40 A	18 B	1841 ABC	219 A
3	42 A	17 B	1501 BC	177 CD
4	34 AB	29 AB	1412 C	183 BCD
5	24 C	21 B	1335 C	204 ABC
6	34 AB	19 B	2376 AB	188 BCD
7	24 C	29 AB	1355 C	187 BCD
8	34 AB	27 B	1448 C	183 BCD
9	28 BC	20 B	1137 C	164 D
10	36 AB	40 A	1503 BC	163 D

<sup>a</sup>Azide application-planting date: 1974

- |                                |                                   |
|--------------------------------|-----------------------------------|
| 1 = no azide, seeded May 9     | 6 = azide May 9, seeded June 6    |
| 2 = no azide, seeded May 23    | 7 = azide May 9, seeded June 20   |
| 3 = no azide, seeded June 6    | 8 = azide May 21, seeded June 6   |
| 4 = no azide, seeded June 20   | 9 = azide May 21, seeded June 20  |
| 5 = azide May 9, seeded May 23 | 10 = azide June 6, seeded June 20 |

Azide broadcast at 70 kg/ha a.i. and rototilled in. Four replicates/treatment.

<sup>b</sup>Variables: 1) % seedling emergence of 50 carrot seeds, 2) root dieback index (RDI) = area of carrot roots with dieback, 3) soil moisture oven dry weight (ODW), and 4) Pythium population (propagules/g ODW soil). Columns with common letters denote means not significantly different at P=.05 of a LSD test. Columns minus letters had a nonsignificant F-value at P=<.05.

Table 5. The effect of rate of sodium azide applied and carrot cultivar on yield.

Treatment	Rate (kg/ha):	Yield variables <sup>a</sup>						
		Plant wt. (kg)	Plant no.	Normal root no.	Normal root wt. (kg)	Abnormal root no.	Abnormal root wt. (kg)	Total root wt. (kg)
0	7.0 A	57.4 A	38.5 A	3.1 A	18.9	1.2	4.3 A	
	6.8 A	56.8 A	37.1 A	2.9 A	19.7	1.3	4.2 A	
	7.2 A	58.0 A	38.0 A	3.1 A	19.5	1.4	4.4 A	
	5.1 B	36.4 B	18.9 B	1.6 B	17.5	1.5	3.1 B	
Cultivar:	5.8 B	55.0 AB	33.00 B	2.4 B	22.0 A	1.5 A	3.9 AB	
	5.9 B	41.3 C	20.50 C	1.8 C	20.8 A	1.6 A	3.4 B	
	5.9 B	63.8 A	48.19 A	3.6 A	14.9 B	1.0 B	4.5 A	
	7.2 A	52.6 B	34.75 B	2.8 B	17.9 AB	1.1 B	3.9 AB	
	7.8 A	47.9 BC	29.06 BC	2.9 B	18.8 AB	1.5 A	4.4 A	

<sup>a</sup>Each variable represents mean yield/3.5 -m row. Plant wt. = foliage + roots, abnormal roots = forked, stubbed, undersized or rotted. Common letters in columns denote means not significantly different at P=.05 of a LSD test. Columns minus letters had a nonsignificant F-value at P<.05. Treatments were replicated 4 times.



Table 6. The effects of rate of sodium azide applied and sampling date on variables related to Pythium root dieback potential.

Treatment	Variables <sup>a</sup>			
	Seedling emergence (%)	RDI	<u>Pythium</u> (propagules/g)	Soil moisture (%)
Date of sampling:				
pre-azide	24 B	20	3509 A	241 A
seeding	24 B	23	1531 B	247 A
harvest	44 A	25	2241 B	178 B
Rate (kg/ha):				
0	33	27	3132 A	232 A
30	33	25	2377 AB	213 C
60	32	17	2077 B	225 AB
90	28	20	2122 B	217 BC

<sup>a</sup>Variables: 1) % seedling emergence of 50 carrot seeds, 2) root dieback index (RDI), 3) soil moisture oven dry weight (ODW), and 4) Pythium population (propagules/g ODW soil). Columns with common letters denote means not significantly different at  $P = .05$  of a LSD test. Columns minus letters had a nonsignificant F-value at  $P < .05$ . Treatments were replicated 4 times.

Table 7. The effect of application rates of Dowco 269 and PP 395 chemicals used for seed treatment on germination of various carrot cultivars.

Treatment	Germination (%) <sup>a</sup>
Cultivar:	
Danvers 126	76 B
Royal Chantenay	85 A
Spartan Fancy	50 D
Waltham Hi Color	71 C
Rate (mg/kg seed):	
0	72 A
500	74 A
1000	72 A
5000	64 B
Chemical:	
Dowco 269	73 A
PP 395	68 B

<sup>a</sup>Based on a sample of 400 seeds (100 seeds x 4 replicates). Common letters in columns denote means not significantly different at  $P = .05$  of a LSD test.

Table 8. The effect of application rates of Dowco 269 and PP 395 chemicals as seed treatments on yields of various carrot cultivars.

Treatments	Yield variables <sup>a</sup>						
	Plant wt. (kg)	Plant no.	Normal root no.	Normal root wt. (kg)	Abnormal root no.	Abnormal root wt. (kg)	Total root wt. (kg)
Chemical:							
Dowco 269	3.9 B	32.6 B	24.1 B	2.2	8.5	.8	6.7 B
PP 395	4.1 A	38.9 A	29.3 A	2.3	9.5	.9	7.1 A
Rate (mg/kg seed):							
0	3.7 B	34.4	26.7	2.2 AB	7.7	.6 B	2.9 B
500	4.2 AB	36.7	28.0	2.4 A	8.7	.9 A	3.3 AB
1000	4.3 A	37.3	27.5	2.5 A	9.7	.9 A	3.4 A
5000	3.8 B	34.7	24.7	2.0 B	10.0	.9 A	2.9 B
Cultivar:							
Spartan Fancy	2.7 B	29.3 B	23.7 B	1.7 B	5.6 B	.4 B	2.1 B
Waltham Hi Color	4.8 A	37.3 AB	20.0 B	1.8 B	17.3 A	1.8 A	3.6 A
Danvers 126	4.2 A	31.7 B	24.8 B	2.5 AB	6.9 B	.7 B	3.2 A
Royal Chantenay	4.4 A	44.8 A	38.5 A	3.1 A	6.3 B	.5 B	3.6 A

<sup>a</sup>Each variable represents mean yield/ 1.8-m row. Plant wt. = foliage + roots, abnormal roots = forked, stubbed, undersized or rotted. Common letters in columns denote means not significantly different at  $P = .05$  of a LSD test. Columns minus letters had a nonsignificant F-value at  $P < .05$ .

Table 9. The effect of application rates of Dowco 269 and PP395 as soil furrow treatments on yields of various carrot cultivars.

		Yield variables <sup>a</sup>					
Treatment	Plant wt. (kg)	Plant no.	Normal root no.	Normal root wt. (kg)	Abnormal root no.	Abnormal root wt. (kg)	Total root wt. (kg)
<b>Chemical:</b>							
Dowco 269	4.3	29.4	19.6	1.8	9.9	.9	2.8
PP 395	4.4	29.5	19.8	1.9	9.7	1.0	2.9
<b>Rate (kg/ha):</b>							
0	4.1	27.2	17.4	1.7	9.8	1.0	2.7
1	4.1	27.9	18.3	1.8	9.6	.9	2.7
2	4.6	30.5	21.0	1.9	9.5	1.0	2.9
5	4.6	32.2	22.0	2.1	10.2	.9	3.0
<b>Cultivar:</b>							
Spartan Fancy	2.7 D	26.4 B	18.8 B	1.4 B	7.6 B	.5 B	1.9 D
Waltham Hi Color	4.6 B	28.4 B	13.3 C	1.3 B	15.1 A	1.6 A	2.9 B
Danvers 126	3.8 C	24.8 B	17.4 BC	1.6 B	7.3 B	.7 B	2.3 C
Royal Chantenay	4.9 A	38.2 A	29.1 A	2.6 A	9.1 B	.8 B	3.4 A

<sup>a</sup> Each variable represents mean yield/1.8-m row. Plant wt. = foliage + roots, abnormal roots = forked, stubbed, undersized or rotted. Common letters in columns denote means not significantly different at P = .05 of a LSD test. Columns minus letters had a nonsignificant F-value at P < .05.

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APPENDIX II

Screening Carrots for Resistance to

Pythium Root Dieback

Screening Carrots for Resistance to  
Pythium Root Dieback

1. HOST: Daucus carota L. var. sativa D.C., cultivated carrot.
2. DISEASE: Brown root (4)  
Rusty root (7)  
Taproot forking (4)  
Lateral root dieback (1)  
Pythium root dieback (PRD) (1,3,7)
3. CAUSAL AGENT: Several Pythium species can incite PRD on carrots but the main ones are P. irregulare Buisman and P. sulcatum Pratt and Mitchell.
4. DESCRIPTION OF PATHOGEN: (6)
  - 4.1 Appearance in culture:  
Colonies of P. sulcatum are completely appressed and submerged on cornmeal agar (CMA). On potato dextrose agar (PDA), weakly cumulous mats of aerial hyphae initially form several millimeters behind growing colony margins but these later collapse so that the colonies appear completely appressed and homogeneous. Growth rates on PDA are slower than those for most other Pythium spp. On CMA at 24°C, radial growth of P. sulcatum was 20 to 40%

greater than on PDA.

4.2 Microscopic appearance:

4.2.1 Mycelium: aseptate except in older cultures,  
and hyaline

4.2.2 Spore forms:

4.2.2.1 Oogonia and oospores: Oogonia terminal or intercalary, usually spherical to subspherical, rarely irregular, 11-21  $\mu$  diam (avg. 16  $\mu$ ). Smaller spherical swellings sometimes present on oogonial hyphae at various distances below the oogonia. Oospores single, apleurotic, 10-18  $\mu$  diam (avg. 14  $\mu$ ) with walls 0.5 - 1.5  $\mu$  thick. Antheridia usually one to three, monoclinal or diclinal frequently arising from the same hypha as the oogial hypha, often branched. Antheridia clavate to crook-necked to elongate with single to multiple transverse furrows.

4.2.2.2 Sporangia and zoospores: Zoospores rarely produced. Vesicles always arise from filamentous sporangia indistinguishable from vegetative hyphae, usually 30-40  $\mu$  diam, liberating



zoospores which are 9-13  $\mu$  diam when encysted. Zoospores sometimes encyst in vesicles which fail to dehisce.

4.2.2.3 Vegetative hyphae bodies: Globose bodies most common (10-30  $\mu$  diam) but also oblong to peanut shaped (45 x 26  $\mu$ ) and interconnected.

5. SOURCES OF PYTHIUM SULCATUM:

- 5.1 P. H. Williams, Department of Plant Pathology, University of Wisconsin, 1630 Linden Drive, Madison, Wisc., 53706.
- 5.2 Commonwealth Mycological Institute, Kew, Surrey, England.
- 5.3 The American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

6. PATHOGENIC VARIABILITY:

- 6.1 Races: No races or biotypes of P. sulcatum have been reported.
- 6.2 Host range: An isolate of P. sulcatum originally obtained from carrots caused root dieback on celery, carrot, parsnip, parsley, radish and turfgrass (1). Susceptible weed species include lamb's quarter (Chenopodium album) and purslane (Portulaca oleracea).
- 6.3 Pathogenic stability: Little is known about the pathogenic stability of P. sulcatum in nature.

- 6.4 Stability in culture: No loss in pathogenicity occurred after 2 yrs. of storage on CMA at 10°C without transfer.
- 6.5 Mechanisms of variation: P. sulcatum is a homothallic species. Heterocaryosis and somatic recombination have not been reported.

7. INCREASING INOCULUM:

- 7.1 Initial inoculum: actively growing hyphal tips of P. sulcatum on agar.
- 7.2 Increase: Prepare cornmeal-sand mixture (CS) by combining silica sand, cornmeal and water in the ratio 20:1:8 (v/v/v) and autoclaving it at 121°C and 15 lbs. pressure for 60 minutes. Inoculate P. sulcatum agar plugs to flasks of CS and incubate at 24°C for 2 weeks with mixing every 2-3 days. Shake CS cultures together into a plastic bag and mix with steamed muck in a ratio of 1:4. Incubate the cornmeal-sand-muck mixture (CSM) at 24°C for 1 week with daily mixing. Pythium populations (propagules/g) may be determined in the CSM by dilution plating on Ocana and Tsao's medium (5). Dilute the CSM to 1000 propagules/g with steamed muck for use as inoculum.

8. LONG-TERM STORAGE AND RETRIEVAL:

- 8.1 Storage: Pure cultures may be stored on cornmeal agar slants in test tubes or screwcap vials at 5-10°C

for 2-3 years and remain viable and virulent.

8.2 Retrieval from storage: Aseptically transfer agar pieces from storage slants to fresh cornmeal agar in Petri plates.

9. SOURCES OF HOST RESISTANCE:

Slicing type varieties especially cv. Scarlet Nantes possess some resistance to PRD.

10. INOCULATION: Layer CSM inoculum (1000 propagules/g) in the bottom of a stainless steel pan (if Wisconsin soil temperature tanks (2) are used) or flats to a depth of 2-3 cm. Embed two ceramic manometer tubes (Fig. 1) in the inoculum about 20 cm apart and cover with 7 cm of steamed muck. Mark rows with a frame (Fig. 2) and seed carrots 1.5 cm deep and 1 cm apart in rows. If germination is suspected to be poor, incubate seeds on moist filter paper in a Petri dish, determine viability and increase seeding density accordingly. Include one row of cv. Waltham Hi color as a susceptible check in each pan.

11. POST-INOCULATION ENVIRONMENT: At 4 weeks of age, thin seedlings to 3 cm apart in the row. Apply water to maintain a soil matric potential of -50 to -150 mb. Flush manometer lines weekly with freshly boiled water, to purge air bubbles from the system. Maintain the soil temperature at 24°C. If in a greenhouse, natural illumination may be supplemented with fluorescent or

incandescent lighting. No fertilizer or nutrient solutions need to be applied if fresh muck is used for each test.

## 12. QUANTIFYING HOST RESISTANCE :

- 12.1 Symptom development: Damping off may occur soon after emergence and continue up to 4-6 weeks. Plants severely affected by PRD will appear stunted and slightly chlorotic. Roots may show varying amounts of dieback of lateral and taproots, stunting, stubbing and forking.
- 12.2 Disease ratings: If carrots are to receive a vernalization treatment to induce seedstock formation, they may be harvested upon reaching a suitable size. Usually, this is after 16-20 weeks of growth. Visually separate the harvested roots into normal and abnormal groups. Abnormal roots are undersized or malformed (forked or stubbed) while normal roots are of a uniform size and shape. Compare roots between test lines as well as with the Waltham Hi Color control when evaluating degrees of resistance.

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APPENDIX III

Screening Carrots for Resistance to  
Rhizoctonia Crown Rot and Cavity Spot

Screening Carrots for Resistance to  
Rhizoctonia Crown Rot and Cavity Spot

1. HOST: Daucus carota L. var. sativa D.C., cultivated carrot.
2. DISEASE: Rhizoctonia crown rot and cavity spot (2,3).
3. CAUSAL AGENT: Rhizoctonia solani Kühn (anastomosis group AG-2).
4. DESCRIPTION OF PATHOGEN: (4)
  - 4.1 Appearance in culture: The fungus grows well on most culture media. On potato dextrose agar (PDA), the mycelium is initially colorless, becoming tan to dark brown when older.
  - 4.2 Microscopic appearance: Young hyphal branches are inclined in the direction of growth and constricted at the junction with the main hypha, but as they grow older, they assume a right angled relation to the latter. In older cultures, the mycelium becomes tufted, dividing into short ovate cells and eventually forming brown sclerotia. The mycelium is abundant and septate at all stages of growth. The perfect (basidial) stage is rarely, if ever, formed in agar culture.
5. SOURCES OF RHIZOCTONIA SOLANI:
  - 5.1 P. H. Williams, Department of Plant Pathology, Uni-

versity of Wisconsin, 1630 Linden Drive, Madison, Wisc., 53706.

6. PATHOGENIC VARIABILITY:

6.1 Races: No races or biotypes of the R. solani inciting crown rot and cavity spot have been reported.

6.2 Host range: R. solani is reported to attack a wide range of vegetable, field and ornamental plants, however, little is known about the virulence of isolates from carrots or other crops.

6.3 Stability in culture: No loss in pathogenecity occurred after 3 years of periodic transfers on PDA and storage at 5-10°C. Loss in pathogenicity was observed after storage on PDA under oil for 2 years.

6.4 Mechanisms of variation: Mechanisms of variation in R. solani include irregular nuclear division in somatic and sexual cells, heterokaryosis, sexual recombination and mutation. Parasexual recombination has not been reported.

7. INCREASING INOCULUM:

7.1 Initial inoculum: Actively growing hyphal tips on cornmeal agar (CMA) or PDA.

7.2 Increase: Aseptically transfer agar plugs from storage to fresh Petri plates of PDA or CMA, then inoculate autoclaved corn kernels (100g corn + 50 ml water/500 ml flask) and incubate at 24°C for 2 weeks with mixing every 2-3 days.



8. LONG-TERM STORAGE AND RETRIEVAL:

8.1 Storage: Store in pure culture on PDA or CMA slants in test tubes or screw cap vials. The fungus remains viable and virulent on agar at 5-10°C without transfer for at least 2 years.

8.2 Retrieval from storage: Aseptically transfer an agar pieces from slants to fresh PDA or CMA and incubate at 24°C.

9. SOURCES OF HOST RESISTANCE:

Little is known about the sources and inheritance of resistance to R. solani in carrots.

9.1 Cultivars: Hi Color 9 (Asgrow Seed Co., Kalamazoo, Mich.) has shown some resistance in field and greenhouse seedling tests.

9.2 Plant introductions and experimental lines: San Nai, a Japanese accession, and 9541, an experimental line, have shown good resistance to R. solani as have the F<sub>1</sub> of crosses of these two. San Nai is available from The Regional Plant Introduction Station, Ames, Iowa as P.I. 226043, while 9541 can be obtained from Dr. L. Baker, Department of Horticulture, Michigan State University, East Lansing, Michigan 48823.

10. GROWING HOST FOR INOCULATION:

Dispense steam sterilized muck into stainless steel pans (if Wisconsin soil temperature tanks (1) are used) or flats

to a depth of 10 cm. Bury ceramic tubes from manometers (Fig. 1) 7 cm below the surface and 20 cm apart. Mark rows with a frame (Fig. 2) and seed carrots thickly enough to allow for thinning to 1 cm apart at 3 weeks of age. Initial seeding density may be judged by testing seed germination on moist filter paper in Petri plates beforehand. Include a row of cv. Royal Chantenay in each pan as a susceptible check.

11. INOCULATION:

Four weeks after seeding, make a furrow 1 cm away from carrot crowns down both sides of each row with a beveled board (Fig. 1), spread a solid line of Rhizoctonia-infested kernels 1.5 cm deep in the furrows and cover with soil.

12. POST-INOCULATION ENVIRONMENT:

Add sufficient water to maintain a soil matric potential of -50 to -150 mb. Flush manometer lines weekly with freshly boiled water to purge air bubbles from the system. Maintain the soil temperature at 24°C. If in a greenhouse, natural illumination may be supplemented with fluorescent or incandescent lighting. No fertilizer or nutrient solutions need to be applied if fresh muck is used for each test. Apply chemicals for the control of foliar insect pests as needed.

13. QUANTIFYING HOST RESISTANCE:

13.1 Symptom development: Crown rot symptoms usually

begin to appear about 2 weeks after inoculation. The collapse of older leaves may be followed by wilting, chlorosis and death of tops due to a rotting of the crown at or below the soil line. Large numbers of plants will usually die from this phase of the disease by 4-5 weeks after inoculation. Thereafter, most other plants will die from cavity spot and a subsequent rotting of the roots rather than of the crown. Dark brown lesions often extending deep below the root surface especially at the point of origin of lateral roots are typical cavity spot symptoms.

- 13.2 Disease ratings: If carrots are to receive a vernalization treatment to induce seedstock formation, they may be harvested upon reaching a suitable size. Usually, this is after 16-20 weeks of growth. Visually separate the harvested roots into diseased and healthy groups. Diseased roots are those which have crown rot or cavity spot, or are markedly undersized or misshapen. Healthy roots are free of rot and of uniform size and shape. Compare roots among test lines as well as with the Royal Chantenay control when evaluating degrees of resistance.

LITERATURE CITED

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MERCURY MANOMETERS (for monitoring soil moisture)

Soil moisture may be continuously monitored with LARK manometer units (Soil Moisture Equip. Corp., Box 30025, Santa Barbara, Calif., 93105).

ASSEMBLY

Cement manometer lines (2 mm O.D.) into 1 m lengths of Nulo-Seal plastic tubing (Imperial Eastman, 6300 W. Howard St., Chicago, Ill. 60648) (2 mm I.D.) which in turn are cemented into 10 cm long porous ceramic tubes (Selas Flotronics, Drescher, Pa. 19025) with a pore size of 0.8  $\mu$  and a bubbling pressure of 12 p.s.i. (3.2 mm I.D.) (Fig. 1). Attach a 30 cm length of Nulo-Seal tubing with a Swagelok brass fitting (Crawford Fitting Corp., 29500 Solon Rd., Cleveland, Ohio 44139) into the other end of the ceramic tube. The brass fitting can be opened by removing the cap but provides an airtight seal when closed.

MAINTENANCE

Use a hypodermic syringe to introduce boiled, deionized water into the manometer lines through the opened fitting. Boiling the water removes dissolved air and helps avoid the formation of air bubbles in the lines. After each use, wash the ceramic tubes and lines free of soil and disinfect them by immersion in 10% formalin for 2-4 hours. Inspect all cemented joints for cracks and leaks and repair these before reuse.

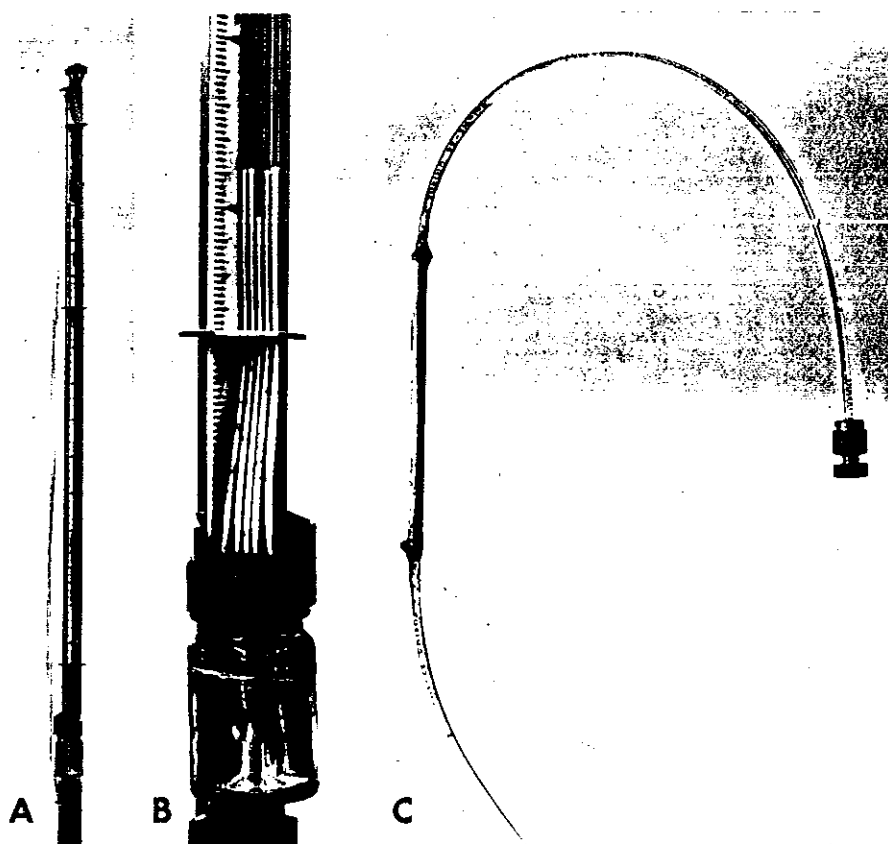


Figure 1. A LARK mercury manometer used to measure soil matric potential during carrot root disease screening. A) Manometer support, scale, lines and mercury reservoir; B) Mercury level indicating a potential of -110 mb; C) Selas ceramic tube, Nulo-Seal tubing and Swagelok brass fitting.