Association of *Pythium* spp. with Carrot Root Dieback in the San Joaquin Valley of California

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ABSTRACT

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Pythium irregulare and *P. ultimum* were frequently isolated from California carrot-producing soils with histories of carrot root dieback. In growth chamber studies, both species caused dieback symptoms and up to 80% mortality of carrot seedlings 7 days after sowing in infested soil. High soil temperatures (27–35 C) aggravated the disease. Saturated soils exacerbated root dieback caused by *P. ultimum*, although soils at −30 kPa matric suction (approximately 11% w/w moisture content) did not limit infection by the fungus. A survey of 39 fields in the San Joaquin Valley in 1987 showed no correlation between incidence of dieback (1–20%) and population densities of *P. irregulare* and *P. ultimum* (0–308 total cfu/g of soil) or between incidence of dieback and a number of soil factors (pH, electrical conductivity, moisture-holding capacity, organic matter, total and exchangeable calcium, and particle size distribution).

Forking and stubbing of mature taproots reduce the quality of carrots (*Daucus carota* L.) produced in California's San Joaquin Valley, where nearly 3,000 hectares are planted annually. Occasionally, as many as 80% of the carrots in a field are malformed and unsuitable for the fresh market. Although forking and stubbing of carrots can be caused by any agent that damages the root apex, such as soil compaction or saturation (9,12), these problems have often been attributed to root dieback caused by *Pythium* spp. (1,2,5–7). If the root apex dies when the root is only a few millimeters long, apical dominance is removed and the taproot either fails to elongate (stubbing) or proliferates to form several functional taproots (forking). In severe cases, the root may not recover and the plant dies.

In organic soils throughout North America, *P. irregulare* Buisman and *P. sulcatum* Pratt & Mitchell are the main incitants of carrot root dieback (1,2, 5–7,13). The etiology of root dieback in the San Joaquin Valley, however, may differ from other carrot-producing regions in North America because carrots there are grown in sandy soil and are often heavily irrigated, particularly in the hot, dry summer months. The purpose of this study was to determine the identity, pathogenicity, and relative abundance of *Pythium* spp. in carrot-producing soils in the San Joaquin Valley and to study the effects of temperature and soil moisture content on root dieback caused by *Pythium* spp. isolated from these soils.

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MATERIALS AND METHODS

Pythium spp. were isolated initially from five commercial carrot fields in Kern County, CA, with histories of serious carrot root dieback. Carrot crops were generally rotated with cotton, potatoes, wheat, onions, or garlic. Isolates obtained by dilution plating and baiting of soil samples collected before or soon after carrot harvest from these fields were identified and used for all subsequent laboratory studies. A systematic sampling of 39 fields planted to carrots in August and September of 1987, including the five original fields, was undertaken to quantify *Pythium* spp. in carrot-producing soils and to correlate disease incidence in 1987 and 1988 with *Pythium* population densities.

Isolations from soil. Five soil cores 2 cm in diameter and 20 cm deep were collected at each of five sites in each field systematically sampled at or near planting time. The sampling sites were located near the center of each quadrant and near the center of the field. The five subsamples were bulked at each sample site and each sample was assayed for populations of *Pythium*.

Subsamples of soil from each site were diluted (1:25 or 1:50, w/v) in 0.2% water agar, plated onto three PVPP agar plates (17 g of Difco cornmeal agar, 5 mg of pimaricin, 250 mg of vancomycin, 50 mg of penicillin, and 100 mg of pentachloronitrobenzene in 1 L of water), and incubated in the dark for 36 hr. Colonies of *Pythium* spp. were counted at 24 and 36 hr. A quantity of soil from each sample was oven-dried to obtain the moisture content. After dilution plating, soil samples from each field were bulked and analyzed by the Soil, Water, and Plant Analysis Laboratory, Cooperative Extension, University of California, Davis, for pH, electrical conductivity, organic matter, particle size distribution, total and exchangeable calcium, and moisture retention at −10 and −1,500 kPa. At harvest (January 1988), the incidence of stubbed or forked carrots in an average of 400 carrots was noted in each field.

Isolations from plant material. *Pythium* spp. were isolated directly on PVPP from diseased carrots collected from commercial fields or from seedlings used as bait. For baiting, carrot seed were sown or young seedlings were transplanted into soil (contained in pots standing in saucers of water) from the original five fields. The pots were placed in a growth chamber maintained at 30/20 C (day/night) with a 12-hr photoperiod. The young carrots were harvested 14 days after sowing or 48 hr after transplanting.

Pathogenicity studies. A total of 13 isolates of five *Pythium* spp. isolated from soil or plants was tested for virulence on carrots. Identification of the isolates was made from morphological characteristics of cultures growing on potato-carrot agar and on blades of sterilized grass in water (10,11). Each isolate was grown on maize meal-sand medium (98% washed sand, 2% maize meal by weight with 20% water by volume) for 2 wk at 25 C in the dark. The colonized medium was then mixed with washed, autoclaved sand (1:1, w/w), incubated for 2 days at room temperature, and diluted with 0.2% water agar at 1:50 and 1:200 (w/v) onto PVPP agar to estimate the number of colony-forming units per gram of sand. The inoculum-sand mixture was further diluted with washed, autoclaved sand to obtain a final *Pythium* count of 200 cfu/g of sand. This population density was used in all subsequent experiments. Three hundred cubic centimeters of the infested sand were then gently packed into each 600-ml Büchner funnel fitted with fritted glass plates of fine porosity (Kimax 600F) and were flooded until saturation. After 2 hr of saturation, the funnels were raised above a water reservoir to give the desired suction at the fritted glass plate. Twenty-five surface-disinfested (1 min in 1.0% sodium hypochlorite in a 10% aqueous ethanol solution) and pre-moistened (24 hr) carrot seeds (cv. Pakmor) were pressed into the sand and allowed to grow for 7 days at a constant matric potential of −2.5 kPa. The funnels

were covered loosely with a polyethylene bag and placed in a growth chamber at 25 C with a 12-hr photoperiod. There were four or more replicate funnels per isolate. Noninfested funnels served as the control. At the end of the trial, the total number of live plants was counted and each of these was observed for root necrosis. All plants were plated onto PVPP after careful washing. The test was repeated at least once with most isolates.

Temperature studies. Development of carrot root dieback in relation to temperature was studied in growth chambers. One isolate of *P. aphanidermatum* (Edson) Fitzp., one isolate of *P. irregulare*, and four isolates of *P. ultimum* Trow were grown on maize meal-sand medium and incorporated into a pasteurized (60 C for 30 min) mixture of peat and vermiculite (50:50, v/v). The infested mix was placed in plastic cones (2.5 cm in diameter at the top by 15 cm deep) set in a rack with their bases submerged in 2 cm of water. Each treatment was replicated five times. The cones were placed inside growth chambers maintained at 25 or 35 C with a 12-hr photoperiod. Five carrot seeds were planted in each cone. After 8 days, plants were removed, washed, rated for the presence of root necrosis, and plated onto PVPP. The experiment was repeated with one isolate each of *P. ultimum*, *P. aphanidermatum*, and *P. irregulare*.

A second experiment was conducted to examine the influence of temperature on infection, symptom expression, and yield of mature carrots. Plastic tubes 6 cm in diameter and 35 cm long were inserted into two insulated boxes heated with thermoregulated coils. The boxes were located in a single growth chamber maintained at 15 C with a 12-hr photoperiod. Tbe boxes were kept at 23 or 27 C. Each box held 12 tubes. An isolate of *P. ultimum* was grown on maize meal-sand and mixed into a pasteurized fine sandy loam. Each tube was filled with noninfested, pasteurized fine sandy loam up to 15 cm below the top of the tube. Half of the tubes in each box were then filled with infested soil and the other half with noninfested soil. The tubes were watered by capillarity from the base.

Results were first obtained 14 days after sowing by destructively assaying three tubes from each treatment. After removing the plants by gentle washing, the number of live plants and the number with root tip necrosis were recorded. In addition, all plants were plated onto PVPP. The plants in the remaining tubes were harvested 70 days after sowing and the number of live plants, the number with forked roots, and total plant weight per tube were recorded.

Influence of soil matric potential on infection. *P. ultimum* was grown on maize meal-sand as described above and incorporated into pasteurized fine sandy loam. Büchner funnels with infested or noninfested soil were saturated with water from below and raised above the reservoir to give suctions of 0, −2.5, −20, and −30 kPa. Twenty-five carrot seeds were planted in each funnel and treatments were replicated three times. Seven days after sowing, the plants were removed from the funnels, washed, counted, and rated for root tip necrosis. All plants were plated onto PVPP to verify infection by the pathogen. The experiment was repeated twice with most of the matric suctions.

RESULTS

Incidence of root dieback in the 39 fields was low (1–20%) in 1987 and 1988, and did not correlate significantly with combined population densities of *P. ultimum* and *P. irregulare* (0–308 total cfu/g soil) ($r = -0.20$) or with individual population densities of either *P. ultimum* ($r = -0.05$) or *P. irregulare* ($r = -0.11$).

The minimum and maximum values for the soil characteristics in the 39 fields were: pH, 6.0–7.9; electrical conductivity, 0.90–2.72 millimhos/cm; total calcium, 14.4–57.1 meq/100 g; exchangeable calcium, 4.0–24.6 meq/100 g; moisture content at −10 kPa, 10.4–32.3%; moisture content at −1,500 kPa, 3.1–10.1%; sand, 53–78%; silt, 10–22%; clay, 8–26%; and organic matter, 0.41–1.04%. There were no significant correlations between incidence of dieback and values of any of the soil variables.

Isolations from soil and plant material. Species of *Pythium* recovered from the five fields with carrot dieback histories are shown in Table 1. A total of eight species of *Pythium* were isolated from the 39 fields by the dilution plate method (Table 2). *P. ultimum* and *P. irregulare*, the most abundant species isolated, were recovered from almost all fields. Some of the isolates of *P. ultimum* produced oospores infrequently and grew at temperatures above 35 C. A few unidentified isolates were asexual but resembled *P. ultimum* in other characteristics. *P. ultimum* was the only fungus recovered from feeder roots of mature carrots. No *Pythium* spp. were isolated from mature taproots.

Three species of *Pythium* were isolated from the five original fields by baiting with carrot seedlings. Of these, *P. ultimum* accounted for over 90% of the isolations. *P. irregulare* and *P. vexans* de Bary were recovered occasionally.

Pathogenicity studies. Because there were no significant differences in plant survival or incidence of root dieback among isolates of the same species of *Pythium*, only the means of the pathogenicity tests for each species are presented (Table 3). *P. ultimum*, *P. irregulare*, and *P. aphanidermatum* were highly virulent, whereas *P. vexans* and *P. oligandrum* Drechsler were of low virulence under the conditions of this study. *P. ultimum*, *P. irregulare*, and *P. aphanidermatum* were recovered from 50% or more of the surviving plants by plating the roots on PVPP. Approximately 25% of the surviving plants showed root necrosis. *P. vexans* and *P. oligandrum* were recovered from less than 25% of the surviving plants, and only the occasional plant showed root necrosis.

Temperature and soil matric potential

Table 1. Population densities of *Pythium* spp. isolated from five carrot fields in the San Joaquin Valley by a dilution plate method

	Dried soil (cfu/g)[z]				
Species	**Field 1**	**Field 2**	**Field 3**	**Field 4**	**Field 5**
P. irregulare	67	84	80	10	48
P. ultimum	33	42	104	20	25
P. oligandrum	16	0	96	36	15
P. aphanidermatum	6	0	6	0	0

[z] Each value is the average number of colonies in five soil samples consisting of five soil cores each. Subsamples of soil were diluted in 0.2% water agar and plated in triplicate on a selective medium.

Table 2. *Pythium* spp. isolated from 39 carrot fields in the San Joaquin Valley by a dilution plate method

Species	**Number of fields from which each species was recovered**	**Percent of all isolations[z]**
P. irregulare	37	42
P. ultimum	31	31
P. oligandrum	20	24
P aphanidermatum	6	1
P. spinosum	2	<1
P. vexans	2	<1
P. paroecandrum	2	<1
P. catenulatum	1	<1

[z] Based on the identification of isolates from soil samples from five sites in each field. Subsamples of soil were diluted in 0.2% water agar and plated in triplicate on a selective medium.

Table 3. Stand densities of carrot seedlings grown at −2.5 kPa matric potential in sand infested with *Pythium* spp. isolated from San Joaquin Valley soils

Species	Live plants 7 days after sowing[z] (%)
Noninfested	93 a
P. oligandrum	74 b
P. vexans	66 b
P. ultimum	19 c
P. irregulare	16 c
P. aphanidermatum	16 c

[z] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test. Treatments consisted of 25 seedlings replicated four times. One single isolate each of *P. oligandrum* and *P. aphanidermatum*, three isolates each of *P. vexans* and *P. irregulare*, and five isolates of *P. ultimum* were included in the test.

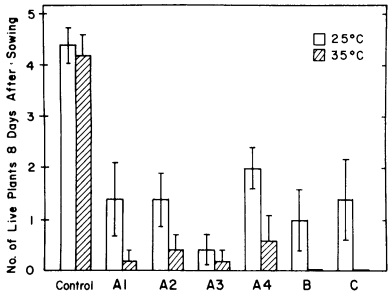

Fig. 1. Influence of temperature on the survival of carrot seedlings grown for 8 days in soil infested with *Pythium* spp. Each bar represents the mean of five replicates. A = four isolates of *P. ultimum*, B = *P. aphanidermatum*, and C = *P. irregulare*.

studies. Significantly more carrots were killed by *Pythium* spp. at 35 C than at 25 C (Fig. 1). At 35 C, *P. irregulare* and *P. aphanidermatum* killed 100% of the seedlings. Similar results were obtained when the experiment was repeated. In the second study, a single isolate of *P. ultimum* killed a similar number of carrots at 23 and 27 C, but at the higher temperature caused a greater reduction in the total weight of the carrots (Table 4). The proportion of forked to normal carrots was also greater at the higher temperature, but root dieback did not result in a significant reduction in the weight of individual carrots.

Infection of carrot seedlings by *P. ultimum* was not inhibited over the range of the experimental soil matric potentials (Fig. 2). In noninfested soil, plant germination and emergence was significantly reduced at saturation (0 kPa). Although *P. ultimum* caused some seedling mortality at all water potentials examined, in saturated soils the fungus killed almost all of the seedlings. Similar results were obtained in the repeated tests.

DISCUSSION

Although soil saturation (12), soil compaction (9), and root apex desiccation

Table 4. Growth of carrot seedlings and root dieback and forking caused by *Pythium ultimum* at two temperatures

Treatment	Temperature (C)	Live plants after 2 wk[x]	Live plants after 10 wk[x]	Forked carrots (%)[y]	Total fresh weight (g)	Fresh weight per carrot (g)
Noninfested	23	9.3 a[z]	10.0 a	10 a	59.29 a	5.66 a
	27	9.3 a	8.7 a	3 a	54.58 a	6.33 a
P. ultimum	23	3.2 b	3.0 b	44 b	21.41 b	5.90 a
	27	3.2 b	2.0 b	83 c	10.66 c	3.69 a

[x] Ten carrots per replicate and three replicates per treatment.
[y] Percent of forked carrots surviving after 10 wk.
[z] Values in each column followed by different letters are significantly different ($P = 0.05$) based on a two-way analysis of variance (three replicates of 10 carrots each).

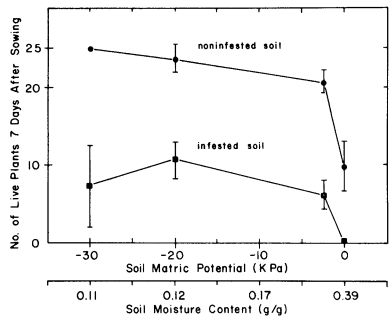

Fig. 2. Influence of soil matric potential on the survival of carrot seedlings grown for 7 days in soil infested with *Pythium ultimum*. The infested soil contained 200 cfu of *P. ultimum* per gram. Treatments were replicated three times.

can cause carrot root dieback, biotic factors probably account for a large incidence of dieback in California; soil fumigation with methyl bromide completely eliminated the problem in a study conducted in Kern County (John Guerard, *unpublished*). Isolation and virulence studies implicate *P. irregulare* and *P. ultimum* as the principal incitants of Pythium root dieback of carrots in the San Joaquin Valley. The association of *P. irregulare* with root dieback appears to be a common factor in the etiology of the disease throughout North America (1,2,6). In contrast, the relative importance of *P. ultimum* in carrot root dieback is primarily a California occurrence. Some of the *P. ultimum* isolates found in San Joaquin Valley soils were unusual in that they produced few oospores on agar or in grass blade-water culture. Oospores that were produced, however, were typical of *P. ultimum*. *P. sulcatum*, one of the primary causal agents of Pythium root dieback of carrots grown on organic soils (1,2), was not isolated from carrots or carrot-producing soils in California. *P. sylvaticum*, another species commonly associated with carrots elsewhere (1,2), was also conspicuously absent despite repeated attempts to cross both of its mating types with the asexual isolates found in our study.

The increased severity of *Pythium*-induced root dieback at high temperatures was consistent with our field observations that root dieback in California usually occurs in summer-sown crops when soil temperatures 15 cm deep in the San Joaquin Valley can reach 39 C (8). The germinability of propagules of *Pythium* spp. that survive the summer may be increased (3), and upon germination encounter a reduced number of antagonists in the warm soils (4). Growers often irrigate newly sown fields twice a day during this time, leading to transient soil saturation that, as our results show, may exacerbate the disease. Our results also show that soil matric potentials less than zero do not necessarily limit root dieback caused by the species of *Pythium* inhabiting carrot-producing soils in the San Joaquin Valley. The isolates of *P. ultimum* and *P. irregulare* studied here were not observed to produce zoospores. The sandy soils in Kern County hold only 39% (w/w) moisture at saturation and a mere 11% (w/w) at −30 kPa suction. Consequently, at least 78% of the available water has drained from the soil as it dries from saturation to −30 kPa suction. Therefore, while the crop is young it is unlikely that the soil would dry sufficiently to restrict infection by *Pythium* because the risk of harming the crop is too great in these soils when they are drier than −30 kPa suction.

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