The Role of Pythium in Feeder Roots of Diseased and Symptomless Peach Trees and in Orchard Soils in Peach Tree Decline

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ABSTRACT

Pythium irregulare was isolated most often from decayed peach rootlets, followed by *P. ultimum* and *P. vexans*. Twelve other *Pythium* spp. were isolated occasionally. No relationship between peach tree decline and occurrence of *Pythium* spp. in peach tree roots or orchard soil was evident. Feeder root necrosis of diseased trees was more than twice that of symptomless peach trees. The number of decayed feeder roots was independent of the number of rootlets invaded by *Pythium* or the number of *Pythium* propagules/g soil. Various levels of *Pythium* in peach orchard soils had no effect on occurrence of peach tree decline during 3 years of observation. *Pythium irregulare, P. vexans,* and *P. ultimum* readily invaded excised dead feeder roots of peach trees, suggesting that these fungi may be saprophytic, secondary invaders of peach tree rootlets. An antibiotic selective medium for measuring population of *Pythium* in soil is described. Phytopathology 61:357-360.

Additional key words: Prunus persica, selective medium for measuring *Pythium* population in soil.

Tree decline or short life of peach (*Prunus persica* [L.] Batsch.) trees is a major and often a limiting factor in several important peach-producing areas. Tree loss has been attributed to specific causes such as *Clitocybe* root and crown rot (14), cold damage (13), bacterial canker (12), plant parasitic nematodes (11), and feeder root necrosis associated with *Pythium* spp. (1, 6).

Poor tree growth, lack of functional feeder roots, and eventual death are common symptoms of peach tree decline. Disease incidence is usually more extensive on land previously planted to peach trees than on newly planted sites. Pre- and/or post-plant soil fumigation results in better growth and longer productive life of trees on sites having a history of peach tree decline or short life of peach trees (5). Thus, soil-borne pathogens may be implicated in this disease.

Hine (7) attributed the peach replant problem to *Pythium ultimum, Fusarium,* and *Rhizoctonia*. Occurrence of *Pythium* spp. in feeder roots and soil was correlated with the peach tree decline problem by Hendrix et al. (6). Peach tree decline in Georgia was attributed to feeder root necrosis caused by *P. irregulare* and *P. vexans* (1, 6); and it was suggested that a *Pythium* population above 50 propagules/g soil may damage peach roots and cause peach decline (5). The experiments reported herein were conducted to elucidate any possible relationship between peach tree decline and occurrence of *Pythium* in peach feeder roots or soil.

MATERIALS AND METHODS.—Soil samples containing roots were collected during April and May under 379 diseased and symptomless peach trees in several mid-Atlantic and southeastern states. Soil under each tree was sampled at three different points within the dripline. At each sampling point, a volume of soil (about $15 \times 15 \times 25$ cm) with the roots was dug. The roots were carefully separated from the soil and examined for discoloration and necrosis, and the percentage of discolored and necrotic feeder roots was visually estimated. Subsamples from under each tree were combined and thoroughly mixed before isolation of *Pythium* was attempted; then the number of propagules per gram of soil (p/g) was determined. *Pythium* spp. were isolated by plating 20 2-cm-long segments of decayed rootlets from each tree on pimaricin-vancomycin selective media (16, 17). The rootlets were surface-sterilized before plating by dipping in 70% ethyl alcohol and drying on paper towels. *Pythium* spp. growing from the rootlets were transferred onto V-8 juice agar containing β-sitosterol (9) and identified.

The population of *Pythium* in peach-orchard soil was determined by sifting soil samples through 0.84-mm mesh screens, preparing soil dilutions of 1:50, 1:100, and 1:200 in 0.3% water agar, and evenly spreading 1 ml onto the solidified selective medium with a slightly bent sterile glass rod. Each sample consisted of three soil dilutions and three plates per dilution.

Several selective media (2, 4, 15, 16, 17) were compared for their efficacy in direct isolation of *Pythium* spp. from peach orchard soil. Since these selective media gave inconsistent results, an improved selective medium was developed that contained pimaricin (Myprozine, potency 92.2%, American Cyanamid Co.), 5 mg; vancomycin hydrochloride (Vancocin, Eli Lilly & Co.) 300 mg; pentachloronitrobenzene (PCNB) technical grade, 100 mg; rose bengal, 10 mg; sucrose, 20 g; Zn ($ZnCl_2$), 1 mg; Cu ($CuSO_4 \cdot 5H_2O$), Mo (MoO_3), Mn ($MnCl_2$), and Fe ($FeSO_4 \cdot 7H_2O$), 0.02 mg each; Mg $SO_4 \cdot 7H_2O$, 10 mg; $CaCl_2$, 10 mg; thiamine hydrochloride, 100 μg; cornmeal agar (Difco), 17 g; agar, 23 g; and demineralized water, 1 liter. Pimaricin, vancomycin, PCNB, and rose bengal were prepared as stock solutions or suspensions in sterile water immediately before adding to the medium. Pimaricin, vancomycin, and $CaCl_2$ (sterilized) were added to the autoclaved (30 min at 15 psi) medium after it cooled to approx 50 C. Fifteen to 20 ml of the medium were poured into each petri dish (100×15 mm) and, as soon as the medium solidified, the dishes were stored in the dark. The medium was prepared and used the same day. Immediately after soil dilutions were spread onto the medium, the plates were incubated for 48 hr at 20 C in the dark. Then the soil was washed from the plates under a slow stream of tap water, and *Pythium* colonies were counted. The plates were incubated for an additional 24 hr to permit slow-growing *Pythium* spp. to become visible. No precaution was required to prevent contamination of the plates by air-borne microorganisms, as the medium does not permit development of these organisms.

RESULTS AND DISCUSSION.—*Qualitative and quantitative determination of Pythium in decayed feeder roots and soil.*—The percentage of decayed and discolored feeder roots of diseased peach trees in the field was 2 to 3.5 times that observed on the symptomless peach trees (Table 1). *Pythium irregulare* Buis. was isolated most frequently from decayed peach rootlets followed by *P. ultimum* Trow and *P. vexans* d By., respectively; in addition, 12 other *Pythium* spp. were occasionally isolated from peach rootlets (Table 1). The average number of rootlets from which *Pythium* was recovered was the same for diseased and symptomless peach trees in Maryland, but it was somewhat greater for symptomless than for diseased trees in Pennsylvania, South Carolina, and Georgia. Only the samples from West Virginia had a higher incidence of *Pythium* associated with rootlets of diseased peach trees than with those of symptomless peach trees. There were no apparent differences in *Pythium* populations in soils collected from symptomless and diseased trees, except in Maryland where the *Pythium* population associated with the diseased trees exceeded that of the symptomless trees. Furthermore, there was no correlation between *Pythium* population in soil and the number of peach rootlets invaded; nor was there any correlation between incidence of *Pythium* in the rootlets and the severity of feeder root necrosis of peach trees. Hendrix et al. (6) reported an average of 69 propagules/g soil in peach orchards with 25% trees affected by peach tree decline, and only 10 propagules/g soil in orchards free of disease in Georgia. Furthermore, they reported that feeder roots of diseased peach trees were almost 3 times more often invaded by *Pythium* than the roots of symptomless trees. The present study showed universal association of *Pythium* spp. with peach trees, and failed to confirm any correlation between *Pythium* population in soil and incidence of the fungus in peach roots or of peach tree decline.

Influence of Pythium population in peach orchard soil on development of peach tree decline.—Attempts were made to evaluate the ability of various levels of *Pythium* population in soil to induce peach tree decline in orchards. An orchard free of peach tree decline in its third growing season at Byron, Ga., was selected for this study. Twelve trees in soil with low (less than 40 p/g soil) and 12 trees in soil with high (over 200 p/g soil) initial *Pythium*-population were observed for occurrence of peach tree decline during a 3-year period. *Pythium* population in soil and the number of feeder roots invaded by *Pythium* were determined for every peach tree each spring and fall. The number p/g soil under trees with the low initial *Pythium* population increased steadily from less than 40 p/g in the spring of 1967 to 339 p/g in the fall of 1969, while the percentage of rootlets from which *Pythium* was recovered declined from 26 to 11% in the same period. *Pythium* population of peach trees with high (over 200 p/g) initial population remained at the original level (200 to 223 p/g) until the fall of 1968; then decreased to 136 p/g in the spring of 1969; and then increased sharply, reaching 701 p/g in the fall of 1969. The number of rootlets from which the fungus was recovered declined steadily from 42% in the spring of 1967 to 2% in the fall of 1969. None of the peach trees in either group developed symptoms associated with peach tree decline, nor was there any observable difference

TABLE 1. Incidence of *Pythium* spp. in rootlets of peach trees and peach orchard soils

State	Condition of tree[b]	% Feeder roots discolored	% Rootlets from which *Pythium* spp. were recovered[a]					No. *Pythium* propagules/g air-dried soil
			P. irregulare	*P. ultimum*	*P. vexans*	*Pythium* spp.[c]	Total	
Maryland	S	35	11	3	1	6	21	231
	D	77	14	1	0	6	21	359
Pennsylvania	S	30	12	11	2	19	44	386
	D	82	15	6	2	14	37	341
West Virginia	S	17	6	13	1	10	30	274
	D	60	17	10	0	25	52	240
South Carolina	S	30	22	5	0	7	34	104
	D	84	22	0	2	7	31	136
Georgia	S	42	36	1	4	5	.46	91
	D	82	23	0	1	8	32	81
Avg[d] for all states	S	31	17	7	2	9	35	217
	D	73	18	3	1	12	34	231

[a] Twenty 2-cm rootlet segments from each tree were plated.
[b] S = Symptomless; D = diseased.
[c] *Pythium splendens* Brown, *P. intermedium* d By., *P. paroecandrum* Drechs., *P. oligandrum* Drechs., *P. monospermum* Pringsheim, *P. rostratum* Butler, *P. mamillatum* Meurs, *P. dissotocum* Drechs., *P. helicoides* Drechs., *P. debaryanum* Hesse, *P. spinosum* Sawada, and *Pythium* spp.
[d] Based on 379 samples: 279 from diseased trees and 100 from symptomless peach trees.

between trees in soil with high and low initial *Pythium* populations. Although it has been suggested that *Pythium* population above 50 p/g soil could cause peach tree decline (5), these investigations failed to confirm this contention. Since peach trees remained symptomless during the 3-year period, even though *Pythium* population exceeded the suggested harmful level of 50 p/g (5) several times, apparently the population of this fungus in soil alone is not reliable in predicting eventual occurrence of peach tree decline in orchards.

Active saprophytic colonization of dead peach rootlets by Pythium.—Several *Pythium* spp. failed to induce feeder root necrosis of 3-month-old peach seedlings in infested, aerated, complete nutrient solution (10) or in artificially infested soil (8). These fungi, however, are readily isolated from dead rootlets of peach trees in the field. *Pythium* spp. may be secondary invaders, and their association with the peach rootlets may be of a saprophytic nature. Several experiments were conducted to determine competitive saprophytic ability of *P. irregulare, P. vexans,* and *P. ultimum* and their ability to invade dead peach rootlets in the presence of soil microflora. Small, excised dead rootlets of peach were mixed with air-dried sandy loam soil free of *Pythium*. The root-soil (1.5:98.5, w/w) mixture was artificially infested separately with each *Pythium* sp. (9) and placed in 8-dr glass vials, and water was added to bring the soil moisture level to 30, 60, and 90% moisture-holding capacity (MHC). The soil water level was maintained throughout the experimental period by placing the vials in a moist chamber. Invasion of dead peach rootlets by *Pythium* spp. was determined after 20 days of incubation at 20 C by plating 20 surface-sterilized rootlets from each vial on pimaricin-vancomycin-PCNB medium (17). The dead rootlets were readily invaded by *Pythium* at all three levels of soil moisture (Table 2). *Pythium vexans* appeared to have the highest competitive saprophytic ability, followed by *P. irregulare* and *P. ultimum,* respectively. High soil moisture favored saprophytic activity of these fungi; thus the number of invaded dead rootlets and the number of p/g were directly proportional to the level of soil moisture. *Pythium vexans,* in particular, showed a high saprophytic activity at low soil moisture (30% MHC), suggesting that predominant recovery of this species from peach rootlets during the summer months (6) is related to its ability to successfully compete, saprophytically, in invading dead peach roots better than do *P. irregulare* and *P. ultimum* at low soil moisture. Apparently *P. irregulare, P. vexans,* and *P. ultimum* behave as soil-inhabiting fungi sensu Garrett (3); they readily invade dead peach rootlets under varied levels of moisture in the soil. Therefore, the association of these *Pythium* spp. with the dead peach rootlets in the field may be of saprophytic nature.

These investigations indicate high incidence of *Pythium* spp. in peach orchards, but do not show any direct relationship between peach tree decline and the occurrence of these fungi in roots or peach orchard soil. This suggests that *Pythium* spp. alone are not the primary cause of peach tree decline; however, the role of *Pythium* spp., in deteriorating feeder roots of peach trees already weakened by other factors, should be further investigated.

TABLE 2. Relative ability of *Pythium* spp. to invade excised dead rootlets of peach trees in artificially infested natural soil at three soil moisture levels

Inoculum	% Soil moisture (MHC)	% Rootlets from which *Pythium* was recovered[a]	No. *Pythium* propagules/g air-dried soil
Pythium irregulare	30	5	500
	60	20	600
	90	45	1,600
Pythium vexans	30	55	5,200
	60	55	7,600
	90	72	11,333
Pythium ultimum	30	8	400
	60	7	400
	90	12	667

[a] Based on two experiments; three replicates/treatment and plating of 20 rootlets/replicate.

LITERATURE CITED

1. BIESBROCK, J. A., & F. F. HENDRIX, JR. 1970. Influence of soil, water, and temperature on root necrosis of peach caused by Pythium spp. Phytopathology 60: 880-882.
2. ECKERT, J. W., & P. H. TSAO. 1962. A selective antibiotic medium for isolation of Phytophthora and Pythium from plant roots. Phytopathology 52:771-777.
3. GARRETT, S. D. 1956. Biology of root-infecting fungi. Cambridge Univ. Press, London & N. Y. 292 p.
4. HENDRIX, F. F., JR., & E. G. KUHLMAN. 1965. Factors affecting direct recovery of Phytophthora cinnamomi from soil. Phytopathology 55:1183-1187.
5. HENDRIX, F. F., JR., & W. M. POWELL. 1970. Control of root pathogens in peach decline sites. Phytopathology 60:16-19.
6. HENDRIX, F. F., JR., W. M. POWELL, & J. H. OWENS. 1966. Relation of root necrosis caused by Pythium species to peach tree decline. Phytopathology 56: 1229-1232.
7. HINE, R. B. 1961. The role of fungi in the peach replant problem. Plant Dis. Reptr. 45:462-465.
8. MIRCETICH, S. M., & H. W. FOGLE. 1968. Occurrence and pathogenicity of Pythium in peach orchard soil. Phytopathology 58:884 (Abstr.).
9. MIRCETICH, S. M., & H. W. FOGLE. 1969. Role of Pythium in damping-off of peach. Phytopathology 59:356-358.
10. MIRCETICH, S. M., & H. L. KEIL. 1970. Phytophthora cinnamomi root rot and stem canker of peach trees. Phytopathology 60:1376-1382.
11. MOUNTAIN, W. B., & H. R. BOYCE. 1958. The peach replant problem in Ontario. VI. The relation of Pratylenchus penetrans to the growth of young peach trees. Can. J. Bot. 36:135-151.
12. PETERSEN, D. H., & W. M. DOWLER. 1965. Bacterial canker of stone fruits in the southeastern states. Plant Dis. Reptr. 49:701-702.
13. SAVAGE, E. F., & F. F. COWART. 1942. Factors affecting peach tree longevity in Georgia. Ga. Agr. Exp. Sta. Bull. 219. 15 p.
14. SAVAGE, E. F., J. H. WEINBERGER, E. S. LUTTRELL, & A. S. ROHDS. 1953. Clitocybe root rot—a disease of economic importance in Georgia peach orchards. Plant Dis. Reptr. 37:269-270.

15. SINGH, R. S., & J. E. MITCHELL. 1961. A selective method for isolation and measuring the population of Pythium in soil. Phytopathology 51:440-444.
16. TSAO, P. H., & J. M. MENYONGA. 1966. Response of Phytophthora spp. and soil microflora to the antibiotics in the pimaricin-vancomycin medium. Phytopathology 56:152 (Abstr.).
17. TSAO, P. H., & G. OCANA. 1969. Selective isolation of species of Phytophthora from natural soil on an improved antibiotic medium. Nature 223:636-638.