

## Pathogenicity of *Phytophthora cactorum* Isolates from New York Apple Trees and Other Sources

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The authors thank H. L. Gustafson for technical assistance, and J. Barnard for advice on statistics and for computer services.

Approved by the Director as Journal Series Paper No. 2153 of the New York State Agricultural Experiment Station, Geneva.

Accepted for publication 1 April 1975.

### ABSTRACT

*Phytophthora cactorum* was isolated from the root crowns of apple trees from two locations in New York State. All trees displayed crown rot symptoms. The isolates were pathogenic to young apple seedlings inoculated with zoospores and also to excised, debarked, apple twigs inoculated with agar plugs bearing mycelium. Differential interaction between apple

cultivars and 15 widely distributed isolates of *P. cactorum* was observed in a twig-inoculation test. Isolates were placed in four groups according to their interactions with six test apple cultivars. The relative susceptibility of 31 important apple cultivars to three *P. cactorum* isolates was determined.

Phytopathology 65:989-994

*Additional key words:* *Malus*, collar rot.

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Syndromes variously referred to as "wet feet", "collar rot", or "crown rot" have been reported as problems of apple trees in New York State for many years (11, 23, 24). *Phytophthora cactorum* (Lebert & Cohn) Schroet., the incitant of similar syndromes in other apple-growing regions (5, 7, 19, 20, 31), has been suspected as a major

cause of the problem, but it has never been directly implicated. Attempts have been made for several years to isolate this organism from affected apple trees without success. The isolations reported here are the first for New York.

Variable pathogenicity, including specialization of

ances of the fungus for particular apple clones, is one possible mechanism for the diverse susceptibility of the same clone reported from different regions (18). Such specialization for different host species has already been reported elsewhere (7, 14, 28). Variable pathogenicity is of particular interest to breeders attempting to develop new cultivars, especially rootstocks, with resistance to *P. cactorum*. Unfortunately, a rapid technique for determining pathogenicity in a natural disease situation is not available. However, the excised-twig method of Borecki and Millikan (6) is useful for determining pathogenicity under specific experimental conditions. It has also been adapted to determine relative tissue susceptibility (6, 10).

The relationship between susceptibility of young seedlings to infection by zoospores and susceptibility of bearing trees has not been thoroughly investigated (30). Therefore, in this study pathogenicity of different isolates of *P. cactorum* was compared in zoospore-irrigated seedling tests and in excised twig tests.

Information on the susceptibility of apple cultivars to infection by *P. cactorum* is incomplete for North American cultivars (1, 2, 9). Such information is of interest to breeders seeking sources of resistance, and also to orchardists wishing to avoid highly susceptible cultivars in regions with favorable conditions for the disease.

**MATERIALS AND METHODS.**—*Isolation of Phytophthora cactorum.*—Isolations were made from small pieces of bark cut from the margins of advancing lesions on the root crowns of apple trees. Two methods of isolation were used successfully. In the first method, pieces of tissue were surface sterilized for 1 minute in 0.5% sodium hypochlorite (10% Clorox), and plated on Tsao and Ocana's P<sub>10</sub>VP medium (25), which contains 10 µg/ml pimaricin, 200 µg/ml vancomycin, and 100 µg/ml pentachloronitrobenzene (PCNB) in Difco cornmeal

agar. In the second method, pieces of tissue were washed in running tap water for 2-3 days. The pieces were flamed, then smaller segments were cut from them and placed in 1% ascorbic acid for 3-5 minutes. These segments were plated on modified Schmitthenner's medium (17), containing 27 mg PCNB, 200 mg vancomycin, 20 mg benomyl, 200 mg yeast extract, 1 g sucrose, 20 g agar, 40 ml V-8 juice filtrate, 960 ml distilled water per liter. After isolation on the selective media, the *P. cactorum* isolates were maintained on V-8 juice agar. Thirteen additional isolates (Table 1) supplied by researchers from other states were also maintained on this medium.

*Inoculation of apple seedlings.*—A modified version of McIntosh's method (1, 13, 30) was used. Agar plugs bearing mycelium of *P. cactorum* were plated on 1.5% lima bean agar plates. After 5 days at 24 ± 2 C the plates were flooded with sterile distilled water to enhance production of sporangia. Two weeks later, the plates were placed in a cold room at -3 C for 20 minutes to induce release of zoospores. The water was poured off and the resultant suspension standardized to 20,000 zoospores per ml using a hemacytometer. Open-pollinated McIntosh apple seedlings were germinated and then planted in a loam:peat:sand mix (1:1:1, v/v) in fiberglass boxes with single drain holes. There were 60 seedlings in each box. When the seedlings had two leaves, the hole was plugged and water added to raise the water table to just above the soil line. A 100-ml aliquot of the zoospore suspension was pipetted along the rows of seedlings in each box. The boxes remained flooded for 48 hours. Some seedlings collapsed and died immediately after the boxes were drained. Others died during the ensuing 2 weeks. Seedlings surviving after 2 weeks were characterized as resistant. In each of two experiments, a box of 60 seedlings was inoculated with each isolate. Some seedlings were flooded with sterile distilled water without zoospores.

TABLE 1. Identity of *Phytophthora cactorum* isolates compared for pathogenicity to apple cultivars

Isolate	Original host	Location	Isolator
NYG-MS2	MM.106 apple rootstock	Victor, N.Y.	H. S. Aldwinckle
NYG-C2	MM.106 apple rootstock	Highland, N.Y.	R. C. Pearson
Mo-1	apple seedling	Lafayette, Ind.	E. B. Williams <sup>a</sup>
Mo-2	M.7 apple rootstock	British Columbia	D. L. McIntosh <sup>a</sup>
Mo-3	M.7 apple rootstock	Illinois	R. Mann <sup>a</sup>
Mo-10	Golden Delicious apple bark	Mt. Grove, Mo.	Z. Borecki and D. F. Millikan
Mo-14	Rome apple tree	Lexington, Mo.)	(Z. Borecki and
Mo-15	Rudy apple tree	New Franklin, Mo.)	(D. F. Millikan
Mo-22 (Zentmyer P285) <sup>b</sup>	Apple	Victoria, Australia	"
Mo-23 (Zentmyer P472) <sup>b</sup>	Pear roots	California	"
Mo-25	Soil around fruiting apple tree	Weston, Mo.	Z. Borecki and D. F. Millikan
Mo-26	Strawberry	California	Wright <sup>a</sup>
Mo-36	Pear fruit	Poland	Z. Borecki <sup>a</sup>
Mo-39	MM.106 apple rootstock	New Franklin, Mo.	D. F. Millikan
ATCC-16695 (Gallegly N95) <sup>c</sup>	Apple trunk		M. E. Gallegly

<sup>a</sup>Obtained from D. F. Millikan.

<sup>b</sup>Original isolate from which Missouri isolate was made.

<sup>c</sup>Original isolate from which ATCC isolate was made.

TABLE 2. Pathogenicity of *Phytophthora cactorum* isolates to young apple seedlings irrigated with zoospore suspensions

Isolate	Seedlings killed <sup>a</sup> (%)
Mo-15	12 x <sup>b</sup>
Mo-39	47 y
NYG-MS2	48 y
NYG-C2	62 y
ATCC-16695	71 y

<sup>a</sup>Mean of two experiments. In each experiment 60 open-pollinated McIntosh apple seedlings were inoculated with each isolate and the percentage of seedlings killed recorded after 2 weeks. No control seedlings irrigated with water alone were killed.

<sup>b</sup>Means not followed by the same letter significantly different ( $P = 0.01$ ), according to Duncan's multiple range test after arcsine transformation.

**Inoculation of excised twigs.**—The method of Borecki and Millikan (6) was followed with slight modifications. Dormant wood of the previous season's growth of 31 apple cultivars was collected in late winter. The sticks were cut into 60-mm internodal lengths and the bark was removed to expose the phloem-cambium region. Four twigs were placed in a petri dish with moistened filter paper on the bottom and cover of the dish. A 4-mm diameter plug was taken from the margin of an actively growing colony of *P. cactorum* on V-8 juice agar and placed in the center of the upper surface of each length of twig. Sixteen twigs of each cultivar were inoculated with each isolate. The twig pieces were incubated in the laboratory at room temperature ( $24 \pm 2$  C) for 8 days, after which the lengths of the resultant brown lesions were measured.

**RESULTS.**—**Isolation of *Phytophthora cactorum*.**—Tissue from the edge of a lesion on the root crown of an apple tree on MM.106 rootstock growing

near Victor, N.Y., yielded a fungus (isolate NYG-MS2) with the morphological characteristics of *P. cactorum* when plated on P<sub>10</sub>VP medium (26, 29). A similar isolate (NYG-C2) from near Highland, N.Y., was obtained on Schmitthener's medium. The trees at both locations were on MM.106 rootstocks in orchards where typical "crown rot" symptoms had been observed. No lesions were observed on scion tissue.

**Pathogenicity of *Phytophthora cactorum* isolates in the seedling test.**—The New York isolates, NYG-MS2 and NYG-C2, were both pathogenic to young seedlings derived from open-pollinated McIntosh apple, a susceptible cultivar (Table 2). NYG-C2 and an ATCC isolate (No. 16695) from apple trunk tissue were the most pathogenic isolates in this test. NYG-MS2 was less pathogenic, equalling Mo-39, an isolate from MM.106 apple rootstock in Missouri. Another isolate, Mo-15, from a Rudy apple tree in Missouri was much less pathogenic. Seedlings were never killed by flooding with sterile distilled water without zoospores.

**Pathogenicity of *Phytophthora cactorum* isolates in the excised twig test.**—Both of the New York isolates were pathogenic to apple phloem-cambium tissue (Table 3). On three of the apple cultivars, NYG-C2 was the most pathogenic of the five isolates assayed in the seedling test. NYG-C2 was also the most pathogenic isolate of the five on the basis of the mean lesion length of all six cultivars. ATCC-16695 was the most pathogenic on three cultivars. Although Mo-15 was not the most pathogenic on any single cultivar, it was almost as pathogenic as NYG-C2 based on the mean lesion length on all cultivars.

The extremes for pathogenicity in the twig test were demonstrated in mean lesion lengths from 14.2 mm to 42.8 mm. On the basis of mean lesion length, the most pathogenic isolates were Mo-25, Mo-22, Mo-2, and Mo-14, none of which was tested for pathogenicity on

TABLE 3. Pathogenicity of *Phytophthora cactorum* isolates to six apple cultivars using excised debarked twigs inoculated with mycelium on agar plugs

Isolate	Length of lesion (mm) <sup>a</sup>						
	Empire	McIntosh	Grimes Golden	Cortland	N.Spy	Baldwin	Mean
Isolates assayed in seedling test:							
NYG-MS2	35.8	25.2	24.0	19.9	18.1	12.0	22.7
NYG-C2	45.2	34.6	28.0	32.3	30.7	12.1	30.5
Mo-15	39.8	42.8	35.1	25.9	28.7	9.1	30.1
Mo-39	43.7	36.3	24.6	14.0	24.9	8.1	25.3
ATCC-16695	46.5	50.8	46.6	11.2	4.0	6.6	27.6
Isolates not assayed in seedling test:							
Mo-1	44.8	33.4	25.2	31.8	32.9	29.0	32.9
Mo-2	56.4	44.4	43.5	34.3	43.3	18.6	40.6
Mo-3	24.1	25.1	28.2	27.6	19.8	6.1	21.9
Mo-10	34.2	27.6	14.3	11.4	19.0	4.1	18.5
Mo-14	54.4	41.4	33.2	48.9	37.6	19.6	39.2
Mo-22	50.3	40.8	52.4	43.3	34.3	32.1	42.1
Mo-23	44.0	33.4	21.6	26.2	27.1	8.1	26.7
Mo-25	57.1	46.9	53.9	33.2	41.3	24.7	42.8
Mo-26	28.4	18.5	13.1	7.0	10.9	7.2	14.2
Mo-36	39.1	28.1	13.9	15.9	21.8	4.8	20.7
LSD ( $P = 0.05$ ) = 8.2 <sup>b</sup>							
F ( $P \leq 0.01$ ) = 5.2 (70, 1,312 d.f.)							

<sup>a</sup>Eight days after inoculation, means of 16 replicates.

<sup>b</sup>This LSD (8, 22) may be used to compare any two figures in the table except for the row means in the last column.

seedlings. These were isolated from apple tissue or from soil around apple trees in Missouri, British Columbia, and Australia, respectively. The least pathogenic isolate, Mo-26, was originally isolated from a strawberry plant in California. The other isolates, of intermediate pathogenicity, were from apple (Mo-1, Mo-3, and Mo-10) or pear (Mo-36) tissue.

Eleven isolates were pathogenic on apple cultivar Empire, two on cultivar McIntosh, two on cultivar Baldwin and one isolate each on cultivars Northern Spy, Cortland, and Grimes Golden.

There were statistically significant differential interactions (8, 22, 27) between apple cultivars and *P. cactorum* isolates in the excised twig test (Table 3). Isolates were placed in four groups on the basis of this differential pathogenicity to the six cultivars (Table 4). If pathogenicity is equated with lesion length, then 11 isolates (Group 1) were most pathogenic on Empire, and, in decreasing order, McIntosh, Grimes Golden, Cortland, Northern Spy, and Baldwin. Statistically significant variations from this order were produced by Mo-22 (Group 2), which induced longer lesions on Grimes Golden than on McIntosh; Mo-14 (Group 3), which induced longer lesions on Cortland than on Grimes Golden; and Mo-2 and Mo-39 (Group 4), which induced longer lesions on Northern Spy than on Cortland.

TABLE 4. Grouping of *Phytophthora cactorum* isolates according to their differential pathogenicity to six apple cultivars in an excised twig test

Group	Order of pathogenicity to test cultivars <sup>a</sup>	Isolates
1	A-B-C-D-E-F	NYG-MS2, NYG-C2, Mo-1 Mo-3, Mo-10, Mo-15, Mo-23, Mo-25, Mo-26, Mo-36, ATCC-16695
2	A-C-B-D-E-F	Mo-22
3	A-B-D-C-E-F	Mo-14
4	A-B-C-E-D-F	Mo-2, Mo-39

<sup>a</sup>Pathogenicity determined by length of lesion induced by isolate on excised twig of cultivar. Decreasing pathogenicity from left to right. Only statistically significant variations from pattern of Group 1 shown. A = Empire, B = McIntosh, C = Grimes Golden, D = Cortland, E = Northern Spy, F = Baldwin.

*Susceptibility of 31 apple cultivars to three Phytophthora cactorum isolates.*—Thirty-one cultivars, including those now producing the most fruit and those presently being planted in the greatest number in North America (3, 4), were assayed for susceptibility to three *P. cactorum* isolates using the excised-twig test. They are

TABLE 5. Susceptibility of 31 apple cultivars to three isolates of *Phytophthora cactorum* in an excised twig test

Cultivar	Length of lesion (mm) <sup>a</sup>			Mean
	NYG-MS2	Mo-15	ATCC-16695	
Baldwin	12.0	9.1	6.6	9.3
Rome Beauty	10.6	9.4	13.6	11.2
Wayne	10.9	13.0	11.7	11.9
Lodi	20.3	6.4	10.7	12.0
Jonamac	11.8	10.9	16.3	13.1
Wellington	19.9	12.0	10.3	14.1
Delicious	12.8	16.6	14.4	14.6
Spigold	29.6	4.7	10.0	14.6
Spijon	21.0	16.8	6.3	14.9
Jonathan	23.1	4.4	17.3	15.9
Golden Delicious	17.4	11.6	20.6	16.5
Tydemans' Early Worcester	13.0	10.6	25.1	16.5
Northern Spy	18.1	28.7	4.0	16.7
Idared	26.4	12.3	9.9	17.4
Monroe	14.9	10.8	25.8	17.4
Mutsu	34.6	10.1	7.9	18.0
Cortland	19.9	25.9	11.2	19.0
Jonagold	16.2	12.3	28.7	19.9
Barry	21.8	17.2	21.6	20.4
Webster	32.2	13.4	16.1	20.7
Milton	28.8	12.3	22.2	21.3
Paulared	17.1	34.3	15.1	22.5
Niagara	18.4	25.1	30.7	24.7
Twenty Ounce	31.9	11.7	33.3	25.3
Macoun	30.7	18.1	27.1	25.5
Spartan	23.6	29.8	28.8	27.4
Starking Golden Delicious	31.3	32.6	21.0	28.3
Grimes Golden	24.0	35.1	46.6	37.0
McIntosh	25.2	42.8	50.8	38.6
Rhode Island Greening	41.1	35.2	42.8	39.7
Empire	35.8	39.8	46.5	40.7

LSD ( $P = 0.05$ ) = 7.8<sup>b</sup>  
 F ( $P \leq 0.01$ ) = 8.1 (60, 1,296 d.f.)

<sup>a</sup>Eight days after inoculation, means of 16 replicates.

<sup>b</sup>This LSD (8, 22) may be used to compare any two figures in the table except for the row means in the last column.

listed in Table 5 in order of their susceptibility, as determined by the mean length of lesions produced in response to all three isolates. Although the length of lesions induced by each isolate tended to increase in this order, there were some significant exceptions.

There were statistically significant interactions (8, 22, 27) between the 31 cultivars and the three *P. cactorum* isolates, which had not been differentiated by six test cultivars (Tables 3 and 5).

**DISCUSSION.**—Attempts over several years to isolate *P. cactorum* from the trunk collars and root crowns of apple trees with the symptoms of "crown rot" in New York State have been largely unproductive. This was in spite of using techniques that others have found successful and that have yielded *P. cactorum* from artificially infected apple seedlings and twigs in the authors' laboratories. This may indicate that the "crown rot" syndrome of apple trees in New York State can be caused by agents other than *P. cactorum*. However, in view of the isolation of pathogenic *P. cactorum* from MM.106 apple rootstocks at two locations in different parts of the state, it is believed that at least on this clone, *P. cactorum* infections contribute significantly to the syndrome. This rootstock has been reported as highly susceptible to *P. cactorum* in British Columbia (12), but resistant in England (18) and New Zealand (21).

The New York isolates were pathogenic both to young apple seedlings irrigated with zoospores and to excised, debarked twigs inoculated with mycelium on agar. With five isolates from New York and elsewhere pathogenicity in the two kinds of test was not correlated significantly although isolate ATCC-16695 was the most pathogenic of five isolates in both the seedling test and on excised McIntosh apple twigs. One isolate, Mo-15, from an apple tree in Missouri, was only slightly pathogenic to seedlings, but caused long lesions on excised twigs. Such differences probably are caused by the different age and physiological state of the tissues challenged, as well as the different inoculation techniques.

The isolates were divided into four groups depending on how they interacted with the six test cultivars (Table 4). Within these groups there were differences in the level of pathogenicity to all cultivars ["aggressiveness" sensu van der Plank (27)]. A larger number of test cultivars would apparently allow further subdivision of the four groups (Table 5). The four pathogenicity groups do not appear to have been determined by host or area of origin. Representatives of groups 1, 3, and 4 had been isolated from apple in Missouri. It is thought inappropriate at this stage to elevate the pathogenicity groups demonstrated here to the status of physiologic or pathogenic races.

These results are apparently compatible with data reported by Sewell and Wilson (18) from experiments on the pathogenicity of four English isolates and one German *P. cactorum* isolates on apple rootstock cultivars. Their data were analyzed to show differences in "aggressiveness" but were not analyzed to show differential interactions (27). The existence in *P. cactorum* of differential pathogenicity to apple cultivars is most critical for the breeder attempting to incorporate resistance in new cultivars. Screening with a representative mixture of several isolates would be desirable.

Of the cultivars now most important in fruit production in North America, McIntosh, Rhode Island Greening, and Grimes Golden, which represent 15% of United States production (4), were very susceptible to the three *P. cactorum* isolates. Cortland and Golden Delicious (15% of U. S. production) were of intermediate susceptibility. Delicious, Rome Beauty, Jonathan, Northern Spy and Baldwin (45% of U. S. production) were less susceptible. Among those cultivars now being planted in New York State (3), Empire was very susceptible; Spartan, Twenty Ounce, Macoun, and Paulared were of intermediate susceptibility; and Mutsu, Tydeman's Early Worcester and Idared were less susceptible. It is unlikely that even the most susceptible cultivars would be at risk because of their own susceptibility unless the stock-scion union was close to the soil line. However, deep planting has been recommended for clonal rootstocks characterized by poor anchorage (16), and if done with low-worked trees will bring the union near the soil line (15).

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