Identification of Pythium ultimum in the Collar Rot of Apple

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ABSTRACT

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Pythium ultimum causes a collar rot disease on apple similar to that caused by *Phytophthora cactorum*. Cultures have been isolated from bark lesions and soil, identified, and their pathogenicity demonstrated.

Additional key words: Malus sylvestris.

In May, 1970, a disease occurred in a trellised planting of apple at the Nowa Weis Station in South Poland. Affected trees had chlorotic foliage, poor growth, and a dark-brown to black lesion at or just below the soil line (Fig. 1), resembling the collar rot disease (1). Twenty-six (13%) of 200 trees (cultivar McIntosh, grafted on EM VII rootstocks) were diseased, and by October they were dead. Other cultivars, including Macoun on EM IV, EM VII, and EM IX roots, and James Grieve, Golden Delicious, and Jonathan on EM IX roots, were affected to a lesser degree. The area in which the trees were planted was poorly drained and exceptionally wet because of heavy late-winter and spring rains. All trees had been mulched annually since 1966 for frost protection.

Fig. 1. Symptoms of collar rot caused by *Pythium ultimum*.

Isolation from the soil, infected bark, and green apples (7) yielded a fungus that appeared to be a *Pythium* species. Two isolates, N from the bark and N-1 from the soil, were selected for further study. When these isolates were grown on malt agar for 48 hours at different temperatures, the optimum growth for N was at 20-25 C and for N-1 at 25-30 C. Both isolates formed a white, luxuriant mycelium after 48 hours at 25 C, with reproductive organs appearing in 4-5 days. After flooding the plates with nonsterilized pond water, both isolates developed many sporangia; those of N-1 were most abundant. Sporangia were spherical and generally terminal (16.3 - 25 μm $\times$ 18.8 - 28.8 μm), frequently intercalary and barrel-shaped (Fig. 2) and germinated directly by a germ tube. Oogonia were spherical, 13.8 - 22.5 μm, generally with a single monoclinous or diclinous antheridium, occasionally both, but never more than two. Antheridia were supported on short pedicels and were slightly curved (Fig. 3). Both N and N-1 were identified as *Pythium ultimum* Trow. according to the descriptions of Middleton (4) and Tompkins et al. (6).

Pathogenicity tests were made in the laboratory by inoculating five 10-cm cuttings of seedling apricot and McIntosh apple with N, N-1 and two isolates of *Phytophthora cactorum* (Leb. & Cohn) Schroet. as described by Sewell and Wilson (5). N and N-1 caused lesions on apricot equalling those of the mildly pathogenic isolate of *Phytophthora*, but were less pathogenic on McIntosh apple.

Three cultivars of apple (McIntosh, Starking Delicious, and Yellow Transparent) were placed in a phytotron on 5 January 1971, and inoculated in triplicate

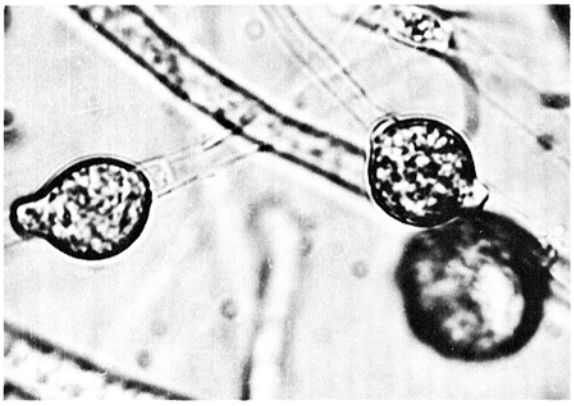

Fig. 2. Spherical terminal, barrel-shaped, and intercalary sporangia of *Pythium ultimum*, isolate N, after 5 days at 25 C.

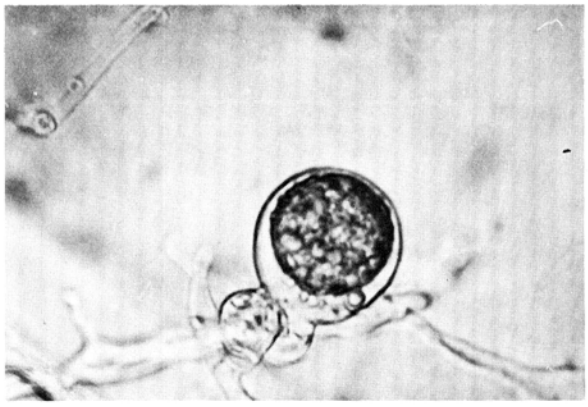

Fig. 3. Antheridium and oogonium of *Pythium ultimum*, isolate N. Note the short, curved pedicel of the antheridium.

TABLE 1. Average lesion size on three cultivars of apple resulting from inoculation with *Pythium ultimum*, cultures N and N-1, and *Phytophthora cactorum*, culture R-15

Apple cultivar	Fungal isolate	Length of lesion (mm) on[x]				
		1/19[y]	2/15[z]	3/18	4/25	6/9
Starking Delicious	N	10 a	28 bg	11 ac	14 def	15 ef
	N-1	11 ac	31 gm	10 a	16 fh	18 h
	R-15	30 g	26 b	39 i	29 g	18 h
McIntosh	N	11 ac	31 gm	10 a	13 cde	12 acd
	N-1	12 acd	12 acd	12 acd	16 f	19 h
	R-15	66 j	68 j	128 k	158 e	34 m
Yellow Trans-parent	N	10 a	28 bg	10 a	13 cde	12 acd
	N-1	10 a	13 cde	19 h	15 ef	15 ef
	R-15	71 j	160 n	83 o	36 i	26 b

[x]Inoculations were made 2 weeks prior to measurements. Means followed by the same letter(s) are not significantly different ($P = 0.05$), using Duncan's multiple range test.

[y]Trees were fully dormant.

[z]Buds were showing green tips.

at approximately monthly intervals with N, N-1, and a highly pathogenic isolate of *P. cactorum* designated R-15 (Table 1). Measurements were recorded 2 weeks after inoculation and showed that the three cultivars reacted to inoculations of N and N-1 in a manner similar to the cultivar reaction (length of lesion) to *Phytophthora* (2, 3). Starking Delicious, which was most susceptible to R-15 in March was most susceptible to N and N-1 in February. McIntosh, which was most susceptible to R-15 in March and April, was most susceptible to N in March, but was rather uniformly susceptible to N-1 throughout the period of these observations. This pattern of susceptibility of McIntosh to N and N-1 was also true for cultivar Yellow Transparent. Although there were these differences in host susceptibility to N and N-1, all three cultivars were susceptible throughout the period of observation.

Field inoculations were made in triplicate on four cultivars (Bancroft, Empire, 0-271, McIntosh) of apple during blossoming when the host is highly susceptible to *P. cactorum* (3). These were inoculated 15 May with two isolates of *P. cactorum* [P-1 (highly pathogenic) and P-18 (mildly pathogenic)] and two *Pythium ultimum* isolates [ATCC 16973 (U-17), and N]. Measurements were made of lesion size 2 months later and reisolations resulted in cultures with typical *Pythium* growth on agar and

TABLE 2. Lesion size resulting from inoculation of four cultivars of apple under field conditions with *Pythium ultimum* isolates, N and ATCC 16973 (U-17) and *Phytophthora cactorum* isolates, P-1 and P-18

Fungal isolate[z]	Length of lesion (mm) on apple cultivars[y]			
	Bancroft	Empire	0-271	McIntosh
N	23 a	39 b	49 c	19 d
U-17	13 e	21 ad	11 e	20 ad
P-1	54 f	45 g	39 b	47 g
P-18	11 e	14 eh	13 eh	15 h

[y]Inoculations were made in triplicate on 15 May 1973, and measurements were made on 15 July 1973. Means followed by the same letter(s) are not significantly different ($P = 0.05$), using Duncan's multiple range test.

[z]P-1 = highly pathogenic, P-18 = mildly pathogenic.

morphological characters described for *P. ultimum* (4, 6).

This study (Table 2) showed that *Phytophthora* isolate, P-1, was the most pathogenic on all cultivars except 0-271, on which N was more pathogenic. Isolate N also was very pathogenic on Empire. The study also showed that the pathogenicity of the type culture, U-17, was comparable to P-18 on Bancroft and 0-271, but its pathogenicity on Empire and McIntosh was greater. Although the pathogenicity of U-17 on Bancroft, Empire, and 0-271 was less than N, it was equally pathogenic on McIntosh.

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