

## The pathogenicity of several *Pythium* species to rootlets of apple seedlings

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### Abstract

From diseased apple rootlets *Pythium sylvaticum* was isolated. Pathogenicity tests have shown that *P. sylvaticum*, *P. ultimum* and *P. intermedium* are strongly pathogenic to the primary root of the apple seedling.

### Introduction

Very little is known about fungal pathogens of apple roots. In fact, apart from the apparent cases of root diseases which lead to clear symptoms on the plant above-ground and eventually to the death of the plant, hardly anything is known about chronic latent root diseases which only take a certain toll of the plant as a whole without leading to clear symptoms.

It has been supposed that the replant disease of apple is caused by pathogenic organisms, but few attempts have been made to identify a specific pathogen causing the chronic diseased condition of the root. Fritsche and Vogel (1954) detected *Phytophthora* oöspores on diseased roots but could not isolate the pathogen. Hoestra et al. (1964) found DD as a soil fumigant to be ineffective against the specific replant disease and thereby excluded nematodes as a cause. The same authors proved chloropicrin to be highly effective.

The literature on *Phytophthora cactorum* as a root pathogen of apple has recently been reviewed by Mulder (1968). Other species isolated from apple rootlets are: *Rhizoctonia solani* (Maciejowska and Williams, 1961), *Pythium ultimum* (Maciejowska and Williams 1961), *Pythium vexans* (Ramakrishnan, 1949) and *Pythium intermedium* (Schultz, 1939). However, *P. aphanidermatum* (Gottlieb and Butler, 1939) and *P. intermedium* (Marchal and Marchal, 1921) were isolated from apple fruits. Extensive research on pear decline in California (Nichols et al., 1964) has revealed the presence of *P. aphanidermatum*, *P. acanthicum*, *P. ultimum*, *P. vexans* and several unidentified *Pythium* spp. in orchard soils.

An attempt was therefore made to isolate a *Pythium* species from apple rootlets collected from soil-sick trees and to study the pathogenicity of various species to apple rootlets.

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## Isolation of a *Pythium* species from apple rootlets

When apple seedlings were grown in soil from a soil-sick apple orchard in test tubes, a rot of the primary bark has been observed (Mulder, 1968) and a white mycelium was seen around the diseased part of the root. On 2% agar substrate a pythiaceous fungus was then isolated. The Centraalbureau voor Schimmelcultures, Baarn, identified this fungus as *Pythium sylvaticum* Campbell et Hendrix, male strain. The same fungus was thereafter isolated from rootlets of an apple tree suffering from replant disease in an orchard. The female form was obtained from a diseased seedling.

*Pythium sylvaticum* was described as a new species by Campbell and Hendrix (1967a, b) occurring in soil under pine, hardwoods, peach, pecans and ornamental shrubs. Filer (1967) proved *P. sylvaticum* to be pathogenic to seedlings of Sweetgum.

## Pathogenicity tests

### *In vitro* tests with *Pythium sylvaticum*

Both male and female strains of *Pythium sylvaticum* proved to be pathogenic to the primary root of apple seedlings. Apple seed was allowed to germinate after 6 weeks of incubation in moist sand at a temperature of 2°C. Seedlings with a root of  $\pm 3$  cm length were placed between a roll of filterpaper and the wall of a test tube. The test tube was filled half with distilled water. The inoculum of *Pythium sylvaticum* in the form of a disk of mycelium on corn meal agar was placed at the root tip. The seedlings were then placed under artificial light at room temperature. Most seedlings (each test consisted of 10 seedlings) developed a rot at or near the root tip. Those that grew past the inoculum were usually not affected any more at a later stage and escaped infection. Under these conditions the fungus was only able to infect the root at or immediately behind the tip.

### *In vitro* tests with other species of *Pythium*

The above-described method for testing the pathogenicity of a fungus to apple seedlings was also applied to the following *Pythium* species:

C.B.S.\*, no. isolated from

<i>P. aphanidermatum</i> (Edson) Fitzp.	339.49	<i>Hibiscus</i> species
<i>P. acanthicum</i> Drechs.	284.31	?
<i>P. debaryanum</i> Hesse	256.38	<i>Beta vulgaris</i>
<i>P. intermedium</i> de By.	267.38	<i>Pinus nigra</i>
<i>P. ultimum</i> Trow	296.37	<i>Pisum sativum</i>
<i>P. vexans</i> de By.	340.49	?

\* List of cultures 1968, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

*P. intermedium* and *P. ultimum* proved to be highly pathogenic, even more so than *P. sylvaticum*. *P. debaryanum* showed some pathogenic capacity, whereas *P. acanthicum* and *P. vexans* were only slightly pathogenic. *P. aphanidermatum* was not pathogenic. The results are shown in Table 1.

Table 1. Pathogenicity of various *Pythium* species to the primary root of apple seedlings. Seedlings planted and inoculated 13.5.68. Record taken 27.5.68.

Species	Number of seedlings	
	healthy	diseased
Control	10	1
<i>P. aphanidermatum</i>	10	0
<i>P. vexans</i>	8	2
<i>P. acanthicum</i>	7	3
<i>P. debaryanum</i>	6	4
<i>P. intermedium</i>	3	7
<i>P. sylvaticum</i> (♂)	3	7
<i>P. ultimum</i>	1	9

Tabel 1. De pathogeniteit van verscheidene *Pythium*-soorten voor de primaire wortel van appelzaailingen. Zaailingen geplant en geïnoculeerd 13.5.68. Waarneming gedaan 27.5.68.

#### *Tests with Pythium spp. in sterilized soil*

Because these findings may have a bearing on the apple replant problem the effect of these fungi on seedling growth in sterilized orchard soil was also tested. Sterilized orchard soil was inoculated with the above-mentioned seven species of *Pythium*. The fungi were grown on a mixture of potting soil and oat meal (5%), sterilized at 120°C during 30 min. The inoculum consisted of  $\pm 50$  g of infested soil thoroughly mixed with the contents of one pot weighing  $\pm 750$  g.

Growth figures are given in Table 2. When considering these figures it must be taken into account that the seedlings differ genetically in growth speed and that several other factors cause considerable variation in growth.

Final records about root infection have not yet been taken but the influence of several *Pythium* spp. on growth of apple seedlings is apparent.

Table 2. Average length (cm) of 10 apple seedlings grown in sterilized orchard soil inoculated with various *Pythium* species. Inoculated and planted 29.4; records taken 11.9.68.

	Average length	S
Control (sterilized soil)	60.5	10.8
<i>P. acanthicum</i>	44.0*	7.8
<i>P. ultimum</i>	41.9*	10.4
<i>P. vexans</i>	40.3**	14.9
<i>P. debaryanum</i>	39.2**	12.3
<i>P. aphanidermatum</i>	39.2**	13.2
<i>P. sylvaticum</i>	34.6**	11.1
Non-sterilized apple orchard soil	29.1**	10.9

\* = significant differing from control ( $P < 0.05$ )

\*\* = very significantly differing from control ( $P < 0.01$ )

Tabel 2. Gemiddelde lengte (cm) van 10 appelzaailingen gekweekt in gestoomde appelboomgaardgrond en besmet met verschillende *Pythium*-soorten. Geïnoculeerd en geplant 29.4- waarnemingen gedaan 11.9.68.

## Conclusion

It may be concluded from these results that several *Pythium* species are pathogenic to apple rootlets *in vitro* and can reduce the growth of seedlings in pot experiments.

## Samenvatting

*De pathogeniteit van verscheidene Pythium-soorten ten opzichte van de kiemwortel van appelzaailingen*

Uit wortels van appelbomen in een perceel waar het verschijnsel bodemmoeheid optreedt, werd de schimmel *Pythium sylvaticum* geïsoleerd. Deze schimmel en ook *Pythium ultimum* en *Pythium intermedium* bleken pathogeen te zijn voor de primaire wortel van de appelzaailing.

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