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Isolation of Phytophthora cactorum from soil in orchards and strawberry fields and differences in pathogenicity to apple

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Abstract

Phytophthora cactorum was isolated from soil of strawberry fields and from an apple and a pear orchard, using hard apples and pears as traps. Pears gave better isolation of the fungus. Isolates from diseased strawberry rhizomes and strawberry-field soil were much less pathogenic to stems and stem and branch pieces of apple variety 'Cox's Orange Pippin' than isolates from apple stem, pear fruit, and orchard soils. Each group of soil isolates showed differences with respect to length and number of the resulting cankers in apple stem and branch pieces.

Introduction

Crown rot of strawberry, caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., was first discovered in the Netherlands in 1967 (Vlasveld, 1968). The strawberry variety 'Glasa' and later also 'Gorella' were severely attacked in particular cases. The disease was originally found in Germany in 1952 in the 'Ydun' variety (Deutschmann, 1954), and also occurs in France (Molot and Nourisseau, 1966) and Belgium (Aerts, 1968).

The fungus *P. cactorum* can cause root, stem and/or fruit rot of many plants (Nienhaus, 1960) and is best known in Dutch fruit growing as the cause of collar rot (Ten Houten, 1958) and crown rot of apple (Van der Scheer, 1969).

It is known from the literature that *P. cactorum* isolates of various plants differ in pathogenicity to a series of hosts. The subject has been reviewed by Nienhaus (1960). Most of the infection trials have concerned the pathogenicity of the isolates to stem and fruit of apple. Only a few trials have been carried out to test isolates from strawberry fruit and rhizomes with respect to their pathogenicity in stem, twig, and fruit of apple, and have led to divergent results (Schmidle, 1957; Braun and Kröber, 1958; Braun and Nienhaus, 1959).

To collect more information on the pathogenicity to stem and stem and branch pieces of apple, the activity of *P. cactorum* isolates from strawberry rhizomes and strawberry-field soils was compared with that of isolates from apple stem, pear fruit, and orchard soil.

Materials and methods

P. cactorum was isolated from the soil by the method mentioned by Roosje (1958, p. 41) and described in detail by Schwinn (1961). Samples were taken from the

upper 20 cm of the soils to be examined and used to fill 2 zinc boxes (50×35×12 cm) each. Fruits of apple and pear varieties were pushed part way into the soil, after which the boxes were kept moist in a greenhouse at 20 °C. When rot on the fruits occurred, a tissue sample was taken from the margin of the spots and transferred to potato-dextrose agar.

In the first trial, soil samples were taken from a pear orchard and a strawberry field on 11 December 1969. In each box of the duplicate zinc boxes filled with these soils, 10 'Golden Delicious' apples and 10 'Beurré Six' pears were placed. For the second trial, soil samples from an apple orchard and the same pear orchard and 4 strawberry fields including the one examined in Trial 1, were taken on 14 January 1970 and kept moist for 2 weeks in the greenhouse at 20 °C, after which each box was baited with 5 'Golden Delicious' apples, 5 'Beurré Six' pears, and 10 'Conference' pears. In the third trial, begun on 13 February 1970, the boxes with soil were the same as those used in the second trial. After the soil had been mulfed 5 'Golden Delicious' apples and 15 'Conference' pears were placed in each box.

Fungus isolates from apple stem, apple rootstock, pear fruit of strawberry rhizome were made in the past by direct isolation from a piece of diseased tissue on agar and then stored at room temperature. The *Phytophthora* spp. were identified by their growth pattern on potato-dextrose agar at 10 °C and 25 °C and the formation and shape of sporangia after floatation of the cultures in tap water.

Inoculation of *P. cactorum* isolates was carried out in stems of 13-year-old apple trees of the variety 'Cox's Orange Pippin' in the field and in about 35-cm-long pieces of stem and branch of 'Cox's Orange Pippin' trees. The diameter of these pieces varied from 4 to 8 cm. The pieces were collected in the winter of 1969/70 and stored at 4 °C before use. One or two days before inoculation, the top ends and wounds were dipped in or smeared with paraffin, after which the pieces were placed in moist peat-dust at 20 °C. In both cases inoculation was made by punching a hole in the bark by means of a cork borer, 8 or 10 mm in diameter, and inserting a piece of potato-dextrose agar containing mycelium into the hole, after which the punch was replaced and sealed with vaseline. The length of the cankers was measured with a marking gauge after removal of the bark.

Stems of 'Cox's Orange Pippin' apple trees were inoculated on 28 July 1969. Five inoculations per isolate were made. Inoculations in stem and branch pieces were made 4 December 1969 and on 26 March 1970. In the first case 20 pieces were used and in the second case 10.

Results

Presence of P. cactorum in soils

The presence of *P. cactorum* in soil of different origin was investigated in 3 trials. The results are shown in Table 1. In the period up to the last check on 22 January 1970, only 3 'Beurré Six' pears in the first trial showed rot due to *P. cactorum*. The rest of the rotted fruits had been attacked mainly by *Phytophthora syringae* Kleb. From trials in foregoing years it was known that *P. syringae* from soil grows more easily in fruits than *P. cactorum* when the temperature is below about 18 °C. Presumably, in the greenhouse the temperature of the soil samples was not raised

Table 1. The presence of *Phytophthora cactorum* in soils of different origin, as shown by isolation from apple and pear fruits, placed on soil samples.

Soil origin	Fruit variety used as trap	Trial 1			Trial 2			Trial 3		
		number of fruits		number of spots caused by *P.cactorum*	number of fruits		number of spots caused by *P.cactorum*	number of fruits		number of spots caused by *P.cactorum*
		total	rotted		total	rotted		total	rotted	
Pear orchard	'Golden Delicious'	20	15	0	10	3	1	10	10	7
	'Conference'				20	20	13	30	30	8
	'Beurré Six'	20	20	0	10	10	3			
Apple orchard	'Golden Delicious'				10	7	0	10	9	6
	'Conference'				20	9	0	30	30	26
	'Beurré Six'				10	10	3			
Strawberry field 1	'Golden Delicious'	20	7	0	10	1	0	10	4	0
	'Conference'				20	18	8	30	30	6
	'Beurré Six'	20	10	3	10	10	0			
Strawberry field 2	'Golden Delicious'				10	0	0	10	5	2
	'Conference'				20	20	8	30	30	14
	'Beurré Six'				10	10	3			
Strawberry field 3	'Golden Delicious'				10	0	0	10	8	0
	'Conference'				20	14	6	30	30	9
	'Beurré Six'				10	10	1			
Strawberry field 4	'Golden Delicious'				10	0	0	10	5	0
	'Conference'				20	8	0	30	29	1
	'Beurré Six'				10	9	0			

Tabel 1. Het voorkomen van Phytophthora cactorum in gronden van verschillende herkomst, aangetoond door isolatie uit op grondmonsters gelegde appel- en perevruchten.

Table 2. Growth of *Phytophthora cactorum* isolates in stem and branch pieces of apple expressed as percentage enlarged cankers of the total number of inoculations and the average length of the enlarged cankers.

Isolate	Source	Trial 1 enlarged cankers		Trial 2 enlarged cankers	
		%	average length (mm) after 38 days	%	average length (mm) after 50 days
a. from plants					
1	'Cox's Orange Pippin' apple stem	100	42		
2	crown of apple rootstock MM 104	100	70	100	27
3	'Précoce de Trévoux' pear fruit	95	45		
4	'Glasa' strawberry rhizome	40	8		
5	'Glasa' strawberry rhizome	35	8		
6	'Glasa' strawberry rhizome	20	7		
7	'Glasa' strawberry rhizome	15	6		
8	'Glasa' strawberry rhizome	35	6	20	8
9	'Senga Pantagruella' strawberry rhizome	50	8		
b. from soil[1]					
A 3 G1	apple orchard			90	29
A 3 G2	apple orchard			80	25
A 3 G3	apple orchard			100	23
P 2 B1	pear orchard			90	18
P 2 B2	pear orchard			100	12
P 2 C1	pear orchard			90	18
P 2 C2	pear orchard			100	13
P 3 G1	pear orchard			100	12
P 3 G2	pear orchard			80	25
P 3 G3	pear orchard			90	18
S1 1 B1	strawberry field 1			20	13
S1 1 B2	strawberry field 1			60	8
S1 1 B3	strawberry field 1			40	9
S1 2 C1	strawberry field 1			20	9
S1 2 C2	strawberry field 1			30	10
S2 2 B1	strawberry field 2			40	9
S2 2 B2	strawberry field 2			10	9
S2 2 C1	strawberry field 2			40	9
S2 2 C2	strawberry field 2			10	10
S2 3 G1	strawberry field 2			10	15
S3 2 B1	strawberry field 3			60	9
S3 2 C1	strawberry field 3			40	11
S3 2 C2	strawberry field 3			40	15
No fungus inoculation (only potato-dextrose agar)			3		2

[1] First column: soil origin (A = apple orchard, P = pear orchard, S = strawberry field); second column: trial number (1, 2, 3); third column: trap (G = 'Golden Delicious', C = 'Conference', B = 'Beurré Six').

Tabel 2. Groei van Phytophthora cactorum isolaties in tak- en stamstukken van appel, weergegeven als percentage gegroeide kankers, alsmede hun gemiddelde lengtegroei.

fast enough. Other trials were therefore carried out with several different soil samples that had been exposed to 20 °C for a longer period before fruits were placed in the boxes.

In the second trial a much higher proportion of the fruits was attacked by *P. cactorum* than had occurred in the first trial and *P. syringae* rot developed much less frequently. It was striking that very few 'Golden Delicious' apples were attacked in the period concerned, i.e. from 28 January 1970 to 13 February 1970.

The third trial was terminated on 10 March 1970 when all the fruits in some of the boxes were rotted. The same amount of *P. cactorum* was isolated from the soil of the pear orchard and the strawberry fields as in the second experiment; but the soil of the apple orchard yielded a much greater quantity of this organism.

P. cactorum rot occurred most frequently in the 'Conference' pears. Only a few 'Golden Delicious' apples were rotted by *P. cactorum*. The fungus was isolated from all soils tested, but the density differed. Collar or crown rot had not been observed in the orchards concerned.

Although all the investigated strawberry fields had shown many diseased plants, in the soil sample from field No 4 only 1 'Conference' pear was rotted by *P. cactorum*.

Inoculation of P. cactorum in stems and stem and branch pieces of apple

The results of the growth of *P. cactorum* isolates in the pieces from 'Cox's Orange Pippin' apple trees are shown in Table 2. The average length of the cankers was corrected to represent the average length minus the punch diameter. Cankers were scored as enlarged only when their length surpassed the average length of the control inoculations with 97% probability. The isolates from strawberry rhizomes were much less pathogenic than the other isolates tested and the resulting cankers showed only minor enlargment after 38 days. The *P. cactorum* isolates from strawberry-field soil showed the same pathogenicity for apple wood as did the isolates from rhizomes of strawberry plants, and the isolates from orchard soils showed the same behaviour as isolates from apple stem and pear fruit. However, the growth differences were less distinct after 50 days. The growth of some isolates from pear-orchard soil was of the same order as that of some of the best-growing strawberry-soil isolates.

The growth of *P. cactorum* isolates was also tested in stems of apple trees in the field. The cankers were measured after 62 days. Five inoculations had been made of each isolate in different stems. Table 3 gives the average length of the cankers

Table 3. Growth of *Phytophthora cactorum* isolates in the stems of 'Cox's Orange Pippin' apple trees.

Isolate[1]	Average length of the cankers (mm) after 62 days
P. cactorum 1	14
P. cactorum 2	53
P. cactorum 3	43
P. cactorum 4	7
P. cactorum 5	7
P. cactorum 6	8

[1] Origin as in Table 2.

Tabel 3. Groei van Phytophthora cactorum isolaties in de stam van 'Cox's Orange Pippin' appelbomen.

Fig. 1. Canker formation by *Phytophthora cactorum* isolate from the rhizome of a strawberry plant (lower arrow) and from the stem of an apple tree (upper arrow) 2 months after inoculation in the stem of a 13-year-old 'Cox's Orange Pippin' apple tree.

Fig. 1. Kankervorming door een Phytophthora cactorum isolatie uit het rhizoom van een aardbeiplant (onderste pijl) en uit de stam van een appelboom (bovenste pijl), 2 maanden na inoculatie in de stam van een 13 jaar oude 'Cox's Orange Pippin' appelboom.

(minus the punch diameter). No real indication of growth of the isolates from strawberry rhizomes could be obtained, because of the lack of control inoculations with potato-dextrose agar, but the other isolates showed distinct growth, as can be seen in Fig. 1.

Discussion

Apple fruits are recommended for the isolation of *P. cactorum* from soil by the method described by Schwinn (1961) and also applied by Aerts (1968). The results of our trials indicate that hard pear fruits, such as unripe 'Conference' are more satisfactory for isolating this fungus. The soil temperature should be high enough to avoid the occurrence of fruit rot by other fungi, especially *P. syringae*. With 'Golden Delicious' apples, *P. cactorum* could be isolated from the soil of only 1 of the 4 strawberry fields, in spite of wide-spread crown rot in the plants. Aerts (1968) reported successful isolation of *P. cactorum* from soil with 'Golden Delicious' fruits when more than 2% of the strawberry plants showed crown rot; his success is probably due to the higher temperature (24 °C) at which he isolated the fungus and the time of the year.

Braun and Kröber (1958) performed inoculation trials with a *P. cactorum* isolate from strawberry roots. No growth occurred when the isolate was inoculated in stems of 'Cox's Orange Pippin' apple trees. Inoculation of 'Cox's Orange Pippin' apple twigs and stems with an isolate from apple tree and strawberry carried out by Braun and Nienhaus (1959) showed that the pathogenicity of the isolate from

strawberry was far lower than that of the apple-tree isolate. Zoospore suspensions of the strawberry isolate gave no infection in the apple stem; mycelium inoculation led to some infection. Nienhaus (1960) reported infection of 'Cox's Orange Pippin' apple stems after inoculation with zoospore suspensions of 3 *P. cactorum* strawberry isolates, but growth of the cankers was much poorer than that of cankers caused by zoospore suspensions of applestem isolates.

The results of our trials confirm the low pathogenicity to apple stems of *P. cactorum* isolates from strawberry rhizome, as compared with isolates from apple stem and pear fruit. Differences in canker growth between the isolates from both soil groups occurred, as was also reported for isolates from apple stems by Ten Houten (1958).

Samenvatting

Isolatie van P. cactorum uit grond van boomgaarden en aardbeivelden en verschillen in pathogeniteit voor appel

De isolatie van *P. cactorum* uit boomgaardgrond en uit grond van aardbeivelden slaagde het beste door gebruik te maken van harde perevruchten. De schimmel groeide uit de grond in de vruchten en kon door overbrengen van een stukje weefsel van de rand van de vruchtaantasting op aardappel-glucose-agar voedingsbodem geïsoleerd worden (Tabel 1). Isolaties uit grond van aardbeivelden waren veel minder pathogeen dan isolaties uit appel- of pereboomgaardgrond, indien ze geïnoculeerd werden in ongeveer 35 cm lange stam- en takstukken van het appelras 'Cox's Orange Pippin', welke in vochtige turfmolm bij 20 °C geplaatst waren (Tabel 2). Evenzo vertoonden isolaties uit aangetaste aardbeirhizomen een geringe pathogeniteit voor stammen en stam- en takstukken van 'Cox's Orange Pippin' in vergelijking met isolaties uit appelstam en perevrucht (Tabel 2 en 3 en Fig. 1). De meeste sterk infektieuse isolaties veroorzaakten grote kankers, doch enkele waren maar weinig virulent.

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References

Aerts, J., 1968. *Phytophthora cactorum* (Leb. & Cohn) Schroet., oorzaak van verwelkingsverschijnselen bij aardbeien. Meded. Rijksfak. LandbWet. Gent 33: 1025–1039.

Braun, H. & Kröber, H., 1958. Untersuchungen über die durch *Phytophthora cactorum* (Leb. u. Cohn) Schroet. hervorgerufene Kragenfäule des Apfels. Phytopath. Z. 32: 35–94.

Braun, H. & Nienhaus, F., 1959. Fortgeführte Untersuchungen über die Kragenfäule des Apfels (*Phytophthora cactorum*). Phytopath. Z. 36: 169–208.

Deutschmann, F., 1954. Eine Wurzelfäule an Erdbeeren, hervorgerufen durch *Phytophthora cactorum* (Leb. et Cohn) Schroet. NachrBl. dt. PflSchutzdienst., Stuttg. 6: 7–9.

Houten, J.G. ten, 1958. Resistance trials against collar rot of apples caused by *Phytophthora cactorum*. Tijdschr. PlZiekt. 64: 422–431.

Molot, P. & Nourrisseau, J.-G., 1966. Dessèchement printanier du fraisier provoqué par *Phythophthora cactorum*. C. r. hebd. Séanc. Acad. Agric. Fr. 52: 1001–1005.

Nienhaus, F., 1960. Das Wirtsspektrum von *Phytophthora cactorum* (Leb. & Cohn) Schroet. Phytopath. Z. 38: 33–68.
Roosje, G. S., 1958. Het mycologisch onderzoek. Jversl. Proefstn Fruitteelt volle Grond 1957: 34–42.
Scheer, H. A. Th. van der, 1969. The occurrence of crown rot caused by *Phytophthora cactorum* in the apple rootstock MM 104. Neth. J. Pl. Path. 75: 369–370.
Schmidle, A., 1957. Über Infektionsversuche an Apfelbäumen mit *Phytophthora cactorum* (Leb. et Cohn) Schroet., dem Erreger der Kragenfäule. Phytopath. Z. 28: 329–342.
Schwinn, F. J., 1961. Der Nachweis von *Phytophthora cactorum* (Leb. et Cohn) Schroet. im Boden. Mitt. biol. BundAnst. Ld-u. Forstw. 104: 42–43.
Vlasveld, W. P. N., 1968. Twee nieuwe ziekten bij aardbeien. Fruitteelt 58: 1018, 1023.

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