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VARIOUS ASPECTS OF PHYTOPHTHORA CACTORUM COLLAR-ROT OF APPLE TREES IN THE NETHERLANDS

Met een samenvatting: Verschillende aspecten van de aantasting van appelbomen in Nederland door Phytophthora cactorum (Leb et Cohn) Schoeter

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INTRODUCTION

Collar-rot of the apple variety Cox Orange Pippin has become a severe disease in several areas of the Netherlands. Orchards in Zeeland and Limburg are most severely affected; in some cases 50–75% of the trees are infected. Since the disease usually appears when the trees are reaching a good bearing age, it represents a considerable financial loss. The disease was first reported in the Netherlands in 1953 by TEN HOUTEN (3). It is known in Germany (2), New Zealand (5), England (6), and Belgium mainly on the Cox variety, and in the U.S.A. (1) on other varieties. In Canada the disease is also found on many varieties but it differs in symptom expression and is known as crown rot (8).

In the Netherlands the disease is being studied cooperatively by Dr J. G. TEN HOUTEN of the Institute for Phytopathological Research, and by several at the Plant Protection Service with the assistance of men at several sub-stations. The author, during ten months of 1954–'55 as guest worker and Fulbright grantee at the Institute for Phytopathological Research, carried out preliminary laboratory and field investigations of the problem which are here presented. Other experiments initiated during this period are now in progress and results will not be obtainable for some time.

THE DISEASE

The disease in the Netherlands is at present of economic importance only on the Cox Orange Pippin variety and the pathogen has been isolated from only one other variety, Allington's Pippin. Rotting of the trunks of other varieties, which is sometimes observed, may be due to the *Phytophthora*, but may also be due to other causes. Trees of the variety Jonathan in one orchard in North Holland appeared to be infected with the *Phytophthora* disease but isolations were not successful.

Cox trees are usually grafted on Malling II, IV, VII, or IX rootstocks with the graft placed a few centimeters below to 20 cm above soil level, usually about half way between. Cankers appear to begin in the swollen tissue a few centimeters above the graft line, and may increase in size to girdle the trunk and reach the scaffold branches. They increase in size more quickly in a vertical than in a lateral direction. No canker has been observed to extend below the graft. Since the Cox

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scion does not usually extend below the soil level, most cankers begin above the soil level, but a few cases were observed where Cox roots had been produced and were infected directly through the soil. Sometimes soil had been ridged around the tree to prevent windthrow and this resulted in infection of the Cox scion. In one orchard Cox „tussenstammen" (intermediate stocks) had been used between Malling rootstocks and Jonathan scions. These Cox „tussenstammen" were invariably infected and the cankers stopped sharply at the rootstocks and extended only very slightly into the Jonathan scion.

The appearance of the rot varies depending upon the time of year and state of activity of the canker. The reddish-brown to brown rot extends to the cambium, and when active, grades into healthy white tissue with intermediate yellowish tissue or with brown flecks beyond a somewhat indefinite margin. Sometimes a ring of mealy white tissue is erupted at the edge of the canker, breaking the epidermis, and dark red droplets appear. When old and inactive, cankered tissue becomes first wet, then soft and is brown (not dark brown) in color; the cankers become sharply delimited when development stops.

Foliar symptoms appear after partial or complete girdling has occurred. Other types of rot may be confused with *Phytophthora* rot; the most similar form is a rot which is dark brown and wet but which does not extend to the cambium. The canker is sharply separated from the healthy white tissue beneath by a thin reddishbrown layer. Another type is a dark brown, dry and hard canker which involves only the outermost tissues of the bark and on which a Pyrenomycete is often fruiting.

It has not been possible to correlate easily observable factors with the occurrence of the disease. In any given orchard infected trees may be scattered at random or 3 or 4 trees in a group may be infected. The watertable in one heavily infected orchard stood at $\frac{2}{3}$ of a meter for much of the year; in another, much lower. Some infected orchards were in grass; some had no cover. All infected trees observed were eight years old or older.

ISOLATION

Successful isolation was difficult but was obtained by first inserting tissue from active cankers into apple fruits, or by very carefully isolating tissue from beyond the brown canker edge onto water or potato dextrose agar.

VARIETAL SUSCEPTIBILITY

An experiment was conducted to compare susceptibility of six varieties by the use of cut branches. Branches 3–4 cm in diameter and 15–20 cm long were inoculated after exposed cuts and the top ends had been dipped in melted paraffine. Inoculations consisted of making a cork borer (5 mm) hole in the bark to the cambium, inserting a piece of agar with the fungus into the hole, and replacing the circle of bark. Vaseline was then placed over the inoculated area to seal the wound. The branches (four for each variety) were stuck into moist sand in pots which were covered with polyethylene bags, and kept in a warm greenhouse. Inoculations were made on November 20th and readings were taken after 45 days.

RESULTS

Large cankers developed only on the Cox variety; cankers on Jonathan, Belle de Boskoop, Yellow Transparent, Manks Codlin and James Grieve were too small and erratic to permit comparison although James Grieve appeared least affected. These results compared well with orchard inoculations carried out on the same varieties in the summer of 1954, but there too the differences between the canker sizes on the various varieties were small and variable except for the reaction on the Cox variety. The results are only tentative for more replications must be used before results can be considered significant. However, it is clear that once present, the fungus can develop much more rapidly in tissue of the Cox variety than in that of the other five.

Results of two other experiments to test varietal resistance showed that inoculations made during the first week of October did not result in sufficient attack on the 50 varieties tested, to permit definite comparisons, although Newton Pippin, Ellisons Orange, Zigeunerin and Lord Lambourne appeared to be most susceptible.

THE PATHOGEN

Twenty different isolates originating from apple cankers in various parts of the Netherlands, Germany and England were examined. All isolates produced similar sporangia and oogonia in several media, and although gross morphology varied slightly, they were typical of *P. cactorum* (LEB. et COHN) SCHROETER. Other isolates originating from Saxifraga, Syringa, Ribes, and pear fruits did not differ significantly in morphology from the apple-canker isolates. Oogonia varied from 24 μ to 35 μ on various media; antheridia were always paragynous; sporangia varied widely from an average of 24 × 35 μ, and had prominent papillae and short stalks.

PEAR- AND APPLE-FRUIT ISOLATES

At least two different *Phytophthora* species may cause fruit rot in the field and in storage in the Netherlands. One is typical *P. cactorum* and the other is a strain resembling *P. syringae* KLEBAHN except for its ability to grow at 25°C.

The *P. syringae* strain may be easily distinguished from *P. cactorum* by the absence of spores on common solid media, its inability to grow at 30°C on 2% potato dextrose agar, and the non-papillate, shallow apical thickening characters of sporangia which are produced in Petri solution. Gross morphology on potato dextrose agar is also different, for *P. syringae* shows regular feather-like sectoring which *P. cactorum* does not produce (Fig. 3). This character is the one first noticed after isolations have been made.

Since the rotting of pear and apple fruits by *Phytophthora* is common in the Netherlands, especially in wet years, it was of interest to know if the fruit-*Phytophthora* could also cause collar-rot.

Pathogenicity of two *P. cactorum* isolates and one *P. syringae* isolate obtained from pear fruits was compared with that of an isolate originating from a collar-rot canker.

Twelve Cox Orange Pippin two-year-old trees were potted, placed in an unheated greenhouse and inoculated on November 15. Each isolate was inoculated

into three trees just above the graft where the trunk was approximately 3–4 cm in diameter. An orchard tree, twelve years old was also inoculated with the four isolates. Results are presented in Table 1.

The striking virulence of isolate No. 28 is immediately apparent. Two of the trees killed by it did not even leaf out in the spring and the cankers extended 20 cm up the trunk. In no case was tissue of the rootstocks (Malling XVI) invaded, even though the scions were rotted to the graft line. The weakness of the attack by the *P. cactorum* isolates is believed to be due to the low temperatures which occurred during the experiment. Isolate No. 28 was able to cause infection even at low winter temperatures, which is characteristic for *P. syringae*.

One of the pear-fruit *P. cactorum* isolates was fully as pathogenic as was the collar-rot isolate, while the other one was slightly but not significantly less so.

TABLE 1. A comparison of pathogenicity of four *Phytophthora* isolates to Cox Orange Pippin trees

TABEL 1. *Vergelijking van de pathogeniteit van 4 Phytophthora isolaties t.o.v. Cox Orange Pippinbomen*

Isolate and source *Isolatie en herkomst*	Young trees *Jonge bomen*		Orchard tree Canker diameter after 3 months *Boom uit boomgaard, diameter van aangetaste plek na 3 mnd*
	Canker diameter [1] after 1 month *diameter van aangetaste plek na 1 maand*	No. killed after 4 months *Aantal, gedood na 4 maanden*	
P. cactorum, pear fruit	2	0	2,6
P. cactorum, pear fruit	3,1	1	2,5
P. syringae, pear fruit No 28 . . .	4,5	3	7,5
P. cactorum, collar-rot	2,8	1	2,1

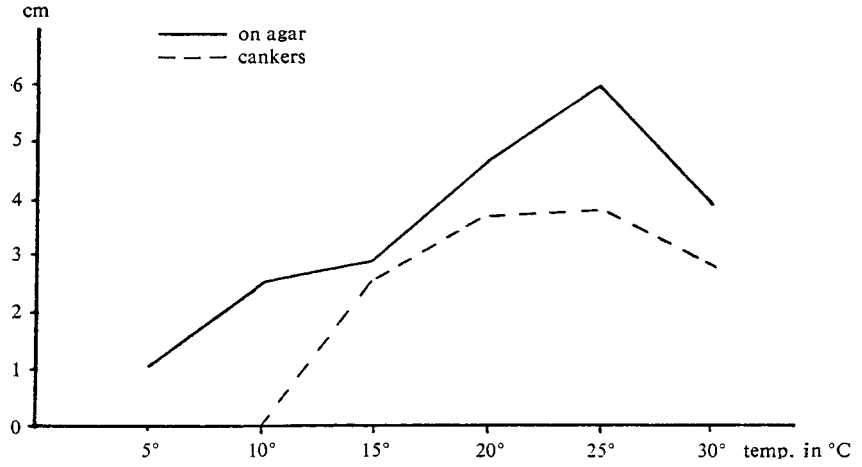
[1]) Length and width in centimeters of cankers on three trees were averaged.
[1]) *Aangegeven is de gemiddelde lengte en breedte in cm van de aangetaste plek per 3 bomen.*

THE RELATIONSHIP BETWEEN TEMPERATURE AND CANKER DEVELOPMENT

Since *P. syringae* No. 28 was so pathogenic at winter temperatures when *P. cactorum* caused little damage, an experiment was carried out to compare canker development at different temperatures. A *P. syringae* isolate from lemon fruit in Portugal was included in the tests to compare it with the similar pear-fruit isolate. The fungi were first grown on potato dextrose agar plates and growth curves were obtained. Then Cox Orange Pippin branches were inoculated, placed in polyethylene bags and incubated at various temperatures. Five replications were used at each temperature. Results are shown in figures 1 & 2 and graphs 1 & 2.

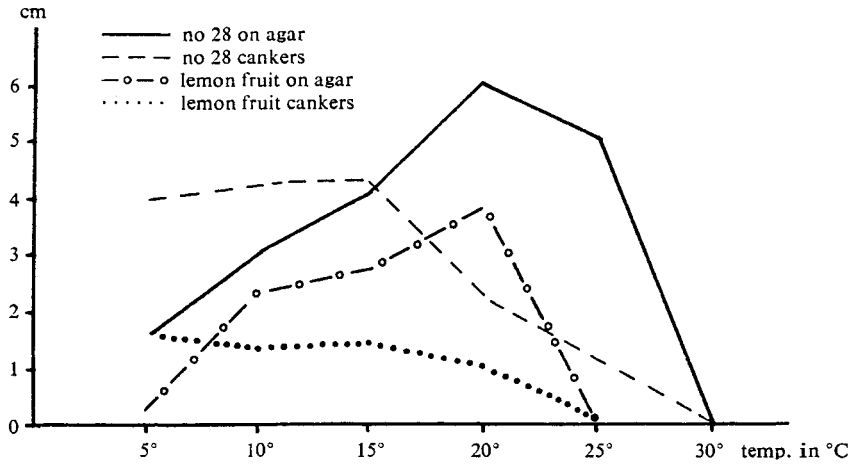
For *P. cactorum* there was a good correlation between growth rate on agar and canker size at the different temperatures, although no cankering occurred at 5° and 10°C. Since the branch substrate is also subjected to differing temperatures, as would occur with the disease in the field, it is felt that the canker size curve is more indicative of the temperatures most favorable to disease development (after infection) than is the growth curve on agar. Temperatures above 15°C and below 30°C, and especially 20–25°C. are favorable for canker-size increase.

For *P. syringae* pear fruit isolate, there was a striking difference between the growth curve on agar and that of canker-size. On agar there was an increase in



GRAPH I. DIAMETERS OF P. CACTORUM COLLAR ROT ISOLATE ON POTATO DEXTROSE AGAR AND CANKER SIZES AT DIFFERENT TEMPERATURES

Grafiek I. Diameter van kolonies van P. cactorum, isolatie uit stambasis op aardappel-dextrose-agar en grootte der rotte plekken op takken bij verschillende temperaturen



GRAPH II. DIAMETERS OF P. SYRINGAE PEAR FRUIT NO. 28 ISOLATE AND LEMON FRUIT ISOLATE ON POTATO DEXTROSE AGAR AND CANKER SIZES AT DIFFERENT TEMPERATURES

Grafiek II. Diameter van kolonies van P. syringae, isolatie nr. 28 van perevrucht en isolatie van citroenvrucht op aardappeldextrose-agar, en grootte der rotte plekken op takken bij verschillende temperaturen

growth to 20 °C., somewhat less at 25 °C. none at 30 °C. Canker-size however was largest and almost constant at 5°, 10°, 15 °C. with a sharp drop to 20° and 25 °C. Although the lemon-fruit isolate of *P. syringae* was only slightly pathogenic, the same sort of curve was demonstrated. On agar this isolate also increased in diameter to 20 °C., but showed no growth at 25 °C. which is characteristic for the species as usually described (7).

Branches were inoculated in the same way with *P. parasitica* DAST. (from Holland), *P. cactorum* (LEB. & COHN) var. *applanata* CHESTER (from Lilac in U.S.A.), *P. hibernalis* CARNE (from orange fruit in Portugal), and with an apple-fruit isolate of *P. syringae* identical in appraearance to the pear-fruit isolate No 28. None produced cankers except the *P. syringae* apple-fruit isolate which produced a large canker, and *P. cactorum* var. *applanata* which produced only a trace of infection.

DISCUSSION

Results of this work showed that *P. cactorum* isolates which occur on apple and pear fruits are readily pathogenic to apple trees and may cause collar-rot after they are inoculated into the trees. Since these isolates are also indistinguishable from collar-rot isolates morphologically and microscopically, it is possible that they play a part in initiating or spreading collar-rot. However it is unknown when infection of the collar takes place; if it is in the late fall, infected apples may function directly in initiating the disease; if it is in the spring or summer, they could only increase the amount of the disease indirectly, through spreading or increasing the inoculum in the soil (unless „early-drop" apples also become infected). Since the canker itself only yields the fungus with difficulty upon isolation, and microscopic inspection rarely reveals evidence of the fungus in canker-tissue, it is believed that inoculum must come from the soil or fruit substrate and not directly from other cankers.

Results showed that a strain of *P. syringae* causing apple- and pear-fruit rot may also cause collar-rot after inoculation, and that at low temperatures it is far more virulent than *P. cactorum*. However, since the *P. syringae* strain has never been isolated from naturally occuring collar-rot it is indicative that there may be factors in the life cycle of this fungus (such as spore production at the right time) which preclude its initiating the disease except when artificially inoculated. On the other hand it may be that it does produce infection but that it is as yet rare and has not been recovered in the small number of isolations made. Temperatures are such in late fall and early winter that, if introduced, it could cause extensive cankering.

The cork-borer method of inoculation and the use of cut branches are useful procedures in studying the disease. However, experiments on intact trees would be more desirable than on cut-branches, especially when higher temperatures are used. At 25° and 30 °C. it is difficult to control drying of cut branches and it is unknown to what extent the lack of a functioning conducting system may affect results.

Although the disease is found only on trees about ten years old or older, trees one and two years old showed no resistance when inoculated. The absence of the disease on young trees may be due to cultural practices, physical factors of the bark surface or to physiological conditions which are different in older trees. Even when young trees were interplanted among older trees in a diseased orchard, they were observed to be not infected. It is possible that the swollen area above the graft, which develops as the trees mature, is especially vulnerable to attack.

Experiments are being conducted to evaluate protective fungicides and fungi-

cides to be applied after the cankers have been cut out. Work is being continued on varietal resistance, on the use of „tussenstammen", and on experiments to establish time of infection. In the meantime certain cultural practices which may be of value in precluding infection are suggested. Mounding around trees to prevent windthrow, which brings soil in contact with the susceptible Cox trunk should be avoided. Where the graft line is below or at the soil surface, the soil should be removed to expose the Cox scion, this will also prevent the production of roots from the Cox scion. Future Cox trees should be grafted as high as possible and if it is not desirable to have the rootstock extend 20–30 cm above the ground, a „tussenstam" should be used. Mechanical wounding of the trunk and irrigation should be avoided.

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SUMMARY

1. *P. cactorum* collar-rot of the Cox Orange Pippin apple variety in the Netherlands is described, and cultural practices favorable to its initiation are mentioned.
2. *P. cactorum* isolates from pear fruits were pathogenic to trunks of the Cox variety, causing damage as extensive as collar-rot isolates.
3. A strain of *P. syringae* capable of growth at 25 °C. was isolated from pear and apple fruits and it was more pathogenic at lower temperatures to trunks of the Cox variety, than were collar-rot isolates of *P. cactorum*.
4. By inoculating cut branches it was shown that the *P. syringae* isolate was most pathogenic at 5, 10, and 15 °C; whereas the *P. cactorum* isolate was not pathogenic at 5 and 10 °C and was most pathogenic at 20, 25 and 30 °C.
5. Lineal growth of three *Phytophthora* isolates on potato dextrose agar at different temperatures was compared with ability to produce cankers at the different temperatures and results are shown in graphs No. 1 & 2 and Fig. 1 & 2.
6. The Cox Orange Pippin variety was shown to be much more susceptible after inoculation than were five other varieties.
7. Isolates of *P. parasitica*, *P. cactorum* var. *applanata*, and *P. hibernalis* were unable to cause measurable infection on Cox branches.
8. A short description is given of the characters of the pear-fruit *P. syringae* isolate and the *P. cactorum* isolates; their gross morphology is shown in Fig. 3.

SAMENVATTING

1. Het stambasisrot bij Cox Orange Pippin in Nederland, veroorzaakt door een aantasting door *Phytophthora cactorum*, wordt beschreven; enige cultuurmaatregelen, die haar optreden begunstigen, worden vermeld.
2. Isolaties van *P. cactorum* uit vruchten van peer konden met succes overgebracht worden op stammen van Cox Orange Pippin-bomen; de schade er

door aangericht was even ernstig als die, welke werd aangericht door isolaties uit de stambasis van Cox Orange Pippin.

3. Een stam van *P. syringae*, die opgekweekt kon worden bij 25 °C, werd geïsoleerd uit pere- en appelvruchten. Zij was bij lagere temperaturen meer pathogeen t.o.v. de Cox Orange Pippin-stammen dan de isolaties van *P. cactorum* uit rotte plekken aan de stambasis van dit appelras.
4. Door afgesneden takken te inoculeren met *P. syringae* kon aangetoond worden, dat de isolatie van *P. syringae* het meest pathogeen was bij 5°, 10° en 15 °C. De isolatie van *P. cactorum* was daarentegen niet pathogeen bij 5° en 10 °C. Zij was het meest pathogeen bij 20°, 25° en 30 °C.
5. De liniaire groei van 3 Phytophthora-isolaties op aardappel-dextrose-agar werd bij verschillende temperaturen vergeleken met hun vermogen om bij deze temperaturen rotte plekken te verwekken. De resultaten zijn weergegeven in de grafieken 1 en 2 en de afbeeldingen 1 en 2.
6. Het ras Cox Orange Pippin bleek bij inoculatie veel vatbaarder te zijn dan 5 andere rassen (Jonathan, Belle de Boskoop, Yellow Transparent, Manks Codlin en James Grieve).
7. Isolaties van *P. parasitica*, *P. cactorum* var. *applanata* en *P. hibernalis* waren niet in staat om op takken van Cox Orange Pippin meetbare infecties te verwekken.
8. Een korte beschrijving wordt gegeven van de kenmerken van *P. syringae*, isolatie uit perevruchten, en van de *P. cactorum* isolaties. Afb. nr 3 geeft de verschillen in groei van deze isolaties weer.

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