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# CROWN ROT OF APPLE TREES IN NOVA SCOTIA[1]

*R.G. Ross and C.O. Gourley*

## Abstract

Crown rot of apple trees caused by Phytophthora cactorum is reported for the first time in Nova Scotia. The extent of the disease is not known but the fungus is widespread in orchard soils. In attempts to isolate P. cactorum by inserting diseased bark into pear fruit a diversity of fungi was obtained. P. cactorum was isolated from the bark of apple trees exhibiting the symptoms of crown rot and was pathogenic to apple bark. Pencillium expansum produced cankers on apple seedlings and detached twigs. Fusarium oxysporum caused cankers on the latter.

## Introduction

In Nova Scotia there have been serious losses in recent years of young apple trees from crown or root troubles which did not appear to be due to unfavorable soil or climatic conditions. No extensive survey of losses was done but in some orchards up to 25% of the trees had died. Most losses occurred in orchards just coming into bearing and included trees on both seedling rootstocks and the Malling series of clonal rootstocks. The syndrome of affected trees was similar to collar or crown rot caused by Phytophthora cactorum (Leb. and Cohn.) Schroet, which has not heretofore been reported on apple trees in Nova Scotia. Many fungi were encountered when attempts were made to isolate P. cactorum from soil and from dying trees.

The fungi isolated and some studies on their pathological characteristics are reported in this paper.

## Isolation of fungi

Attempts to isolate P. cactorum from diseased areas of roots and crowns of apple trees by inserting strips of bark into the flesh of apple or pear fruits were unsuccessful. Numerous rots developed in the fruits and isolations onto potato-dextrose agar (PDA) yielded a diversity of fungi. Bark from 4- to 5-year-old Wayne apple trees on seedling rootstock collected in September 1969 and irrigated in running tap water for 2 days before being inserted into pear fruits yielded Monilinia fructicola (Wint.) Honey (IMI 164415), Botryospheria obtusa (Schw.) Shoem., Fusarium roseum Lk., Botrytis sp., and Monilinia laxa (Alderh. and Ruhl.) Honey. However. bark from these trees collected in October but not irrigated before insertion into pear fruits yielded Alternaria alternata (Fr.) Keissler, Botrytis cinerea Pers., Fusarium oxysporum Schlecht., Trichoderma sp., Fusarium solani (Mart.) App. and Wr. and Cytospora ambiens Sacc. Similarly hark collected in October from 8-to 9-year-old McIntosh trees on MM104 rootstock yielded Phomopsis sp. (stat. perf. Diaporthe eres Nit.), Penicillium expansum Link ex S. F. Gray (IMI 158108), A. alternata, and a bacterium. In June and July 1970, bark from the Wayne and McIntosh trees, which were located in widely separated orchards, and from Spartan apple trees on seedling rootstock in another orchard yielded only P. expansum when inserted into apple fruit.

## Inoculation experiments

In 1969, Phytophthora cactorum was isolated from decayed apples collected from the ground and from apples on lower limbs and in contact with the ground in the Wayne and McIntosh orchards and in several other orchards in which the trees had no apparent crown or root troubles. Subsequently in 1970 soil samples from the infected root and crown zones of the Wayne and McIntosh trees were puddled in shallow pans and apple fruit from the previous year's crop were placed on the surface. P. cactorum was isolated from the soil from the McIntosh orchard but not from the Wayne orchard.

In September 1970, a selective medium (2) was used to isolate P. cactorum from diseased bark. It contained-cornmeal agar (Difco, 17 g/liter) supplemented with pimaricin, penicillin "G"-potassium and polymixin B sulphate at 100, 50, and 50 ppm, respectively. Strips of diseased bark were

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irrigated in running tap water for at least 2 days, and sections plated on the medium. Isolations were attempted from 19 trees of various cultivars on a variety of rootstocks. P. cactorum was isolated from 4 tress. One was a McIntosh on MM104 rootstock in the orchard referred to above, and the other 3 were Greening on MVII rootstock from another orchard. The Wayne trees did not yield P. cactorum.

## Seedlings

On March 31, 1971, dormant I-year-old Beautiful Arcade apple seedlings were removed from storage, their roots washed free of soil, and a lonqitudinal incision about 10 mm long and 2 mm deep was made with a flamed scalpel in the stem just above the roots. Groups of these seedlings were inoculated with P. cactorum from cornmeal agar or with P. expansum or F. oxysporum from PDA by inserting a 3 mm agar plug containing fungus mycelium under the flap of bark which was pressed down and held in place with a single layer of masking tape. Controls consisted of seedlings with a plug of sterile agar medium inserted under the flap and seedlings in which no incision was made. Excess roots were cut off and the seedlings pruned back to about 30 cm of stem. They were then potted in a mixture of soil, peat and sand (1:2:1) plus nutrients in 15 cm clay pots so that the incisions were below the surface of the mixture. The potted seedlings were placed in the greenhouse and watered twice daily.

One month after the initial inoculation, inoculum consisting of agar plugs of P. cactorum was placed over the incision flap of four seedlings that had been inoculated with P. expansum and four that had been inoculated with F. oxysporum. The soil was withdrawn from around the original point of inoculation, the tape removed, a plug of inoculum placed over the incised area, retaped, and the soil replaced. Similarly inoculum of P. expansum and F. oxysporum was placed over the flaps of seedlings initially inoculated with P. cactorum. This procedure was repeated on a different group of four seedlings 2 months after the initial inoculation. Four inoculated seedlings for each fungus were left undisturbed.

Two seedlings initially inoculated with P. expansum and two inoculated with P. cactorum did not leaf out. They were removed and examined 35 days after inoculation. Suckers were coming up from the roots of the P. expansum seedlings but not from the roots of those inoculated with P. cactorum. The inoculated area of one P. expansum inoculated seedling was surrounded by a sunken canker 28 mm in length which almost encircled the stem. Underneath the canker was a concave area of brown decayed tissue extending almost through the stem. The other seedling had a canker 45 mm long which encircled the stem but was not sunken. The two P. cactorum inoculated seedlings had decayed-areas about 40 mm long girdling the stems.

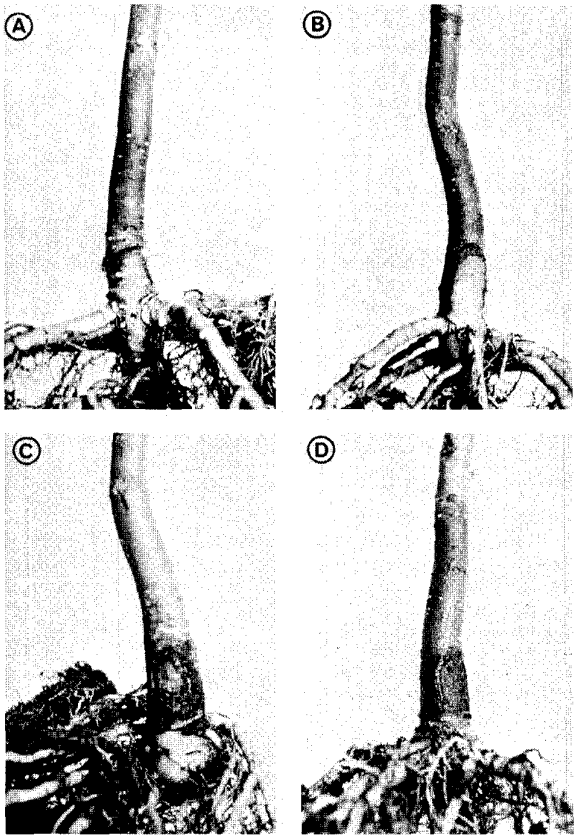


Figure 1. Beautiful Arcade opple seedlings 1 month after inoculation. A) agar plug, B) *Fusarium* oxysporum, C) *Penicillium* exponsum, D) Phytophthoro *cactorum*.

One month after the initial inoculations, damage from P. expansum was more severe than from P. cactorum (Figure 1). P. cactorum cankers were not sunken and usually not extensive, although cankers on a few of these seedlings half encircled the stems. P. expansum cankers were sunken with definite margins and were about 13 mm wide and up to 25 mm lonq. Incisions on the seedlings inoculated with F. oxysporum appeared to be completely healed.

Five months after the initial inoculations all seedlings were removed from the pots and examined. Except for some slight callousing where P. expansum had been placed over P. cactorum inoculations there were no obvious differences between seedlings that had been inoculated with P. cactorum alone and seedlings where at 1 and- 2 months inoculum of P. expansum or F. oxysporum had been placed on P. cactorum cankers. Likewise placing P. cactorum on wood inoculated with P. expansum or F. oxysporum had no obvious effect. Most seedlings inoculated with P. cactorum alone appeared healthy except for a

brown film of dead tissue over previously cankered areas, which readily sloughed off revealing healthy tissue. Where complete girdling had occurred the roots below the canker were usually dead and new roots had developed above the canker. Considerable callus tissue had formed around the deep sunken P. expansum cankers which resembled those already described. Dead wood extended through 2/3 of the stem and the core was brown or discolored for 2 or 3 cm above and below the cankers. Seedlings inoculated with F. oxysporum appeared to be completely healed but on dissection there was often a shallow area of discoloration below the areas of inoculation.

Isolations for P. expansum and F. oxysporum were made on PDA and for P. cactorum on the selective medium (2). Only 3 of the 36 seedlings inoculated with P. cactorum yielded P. cactorum on reisolation. Two were seedlings where P. expansum had been placed over P. cactorum cankers at 1 month and the other was a seedling where P. expansum had been added at 2 months. P. expansum was readily reisolated from the edges of surface cankers and from the internal decayed or discolored areas of all seedlings in which it had been placed. F. oxysporum was recovered from the discolored areas below the inoculation point from about 2/3 of the seedlings inoculated with this organism.

Detached twigs

On January 27, 1971, terminal shoots from dormant apple trees were cut into 14 cm lengths and inoculated by replacing a 3 mm bark disc with a disc of fungus mycelium in the agar medium used in inoculating seedlings (4). Four shoots of each cultivar were inoculated with P. cactorum and one with P. expansum and F. oxysporum. The experiment was repeated on twigs collected April 1, 1971, except that two shoots of each cultivar were used for each of the latter two fungi. Each inoculated twig was placed in a metal capped test tube containing 4 cm of water and incubated at room temperature. Controls consisted of twigs with sterile agar plugs. Four weeks after inoculation the lesion lengths (Figure 2) were recorded and the twigs that had been inoculated with P. cactorum were laid on the surface of the Phytophthora-selective medium. After 4 days the distance along the shoot from which P. cactorum emerged to form a colony was measure . Isolations from P. expansum- and F. oxysporum-inoculated shoots were made on PDA and since sunken cankers with definite margins were formed by these funqi it was possible to measure their length.

On the Phytophthora-selective medium P. cactorum grew from all shoots except two of MM104 inoculated in January. Uninoculated and inoculated shoots of MM104 standing in water became heavily colonized by a variety of fungi whereas shoots of the other cultivars were relatively free of these

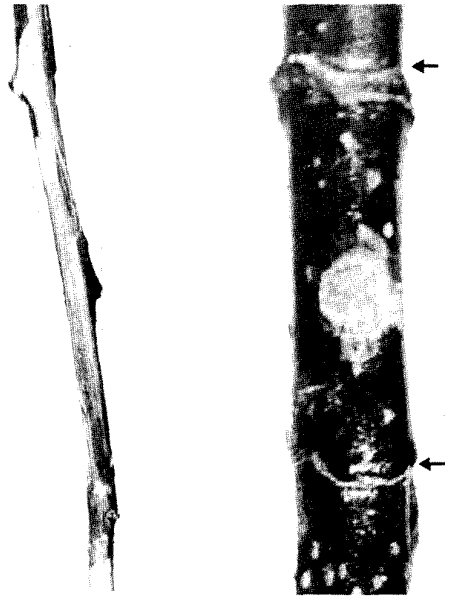

Figure 2. Left – Lesion produced by Phytophthoro coctorum on Beautiful Arcade opple shoot. The bark has been removed to show internal discoloration. Right – Canker produced by *Penicillium* expansum on McIntosh apple shoot (arrows delimit edges of canker).

colonizers. With a few shoots it took longer than 4 days for P. cactorum to emerge and their measurements were not included in the average length of colonies given in Table 1. P. expansum and F. oxysporum were also readily reisolated from the edges of the cankers produced by these organisms and isolations from the lesioned areas which extended in the wood at various distances from the definite sunken cankers were usually positive or the appropriate fungus.

## Discuss on

These investisations show that crown rot of apple trees caused by P. cactorum is present in Nova Scotia. As in British Columbia (5) it appears to be confined to the near ground level portion of the tree. In some areas P. cactorum causes trunk cankers to above ground parts of apple trees (1) but this type of canker has not been identified in Nova Scotia. This preliminary work does not give any indication of the extent of the disease or what proportion of tree losses might be due to P. cactorum. It does, however, show that the fungus is widespread and points out the danger of using rootstocks susceptible to P. cactorum (3).

In attempts to isolate P. cactorum by placing bark samples in pear or apple fruit a number of fungi was obtained. This method obviously isolates only the organisms most aggressive in the fruit. It is interesting that in pear fruits an entirely different

Table 1. Length (mm) of lesions and cankers on apple shoots 4 weeks after inoculation with fungi associated with crown rot

| Cultivar | Phytophthora cactorum | | *Penicillium* expansum | | Fusarium oxysporum | |
|---|---|---|---|---|---|---|
| | Lesion | Colony emergence* | Canker | Lesion | Canker | Lesion |
| | | | January 27 inoculations | | | |
| McIntosh | 81 | 84 | 11 | 10 | 9 | 12 |
| Cortland | 15 | 23 | 6 | 7 | 11 | 16 |
| Red Spy | 48 | 43 | 6 | 8 | 7 | 8 |
| Gravenstein | 79 | 73 | 6 | 8 | 14 | 10 |
| Beautiful Arcade | 100 | 84 | 9 | 9 | 4 | 10 |
| MM 104** | 108 | 30 | 30 | 138 | 13 | 90 |
| | | | April 1 inoculations | | | |
| McIntosh | 125 | 103 | 21 | 111 | 6 | 45 |
| Cortland | 81 | 119 | 20 | 81 | 7 | 8 |
| Red Spy | 123 | 104 | 18 | 76 | 4 | 9 |
| Gravenstein | 44 | 114 | 17 | 140 | 10 | 24 |
| Beautiful Arcade | 67 | 88 | 10 | 76 | 4 | 5 |
| MM 104** | 126 | 110 | 15 | 120 | 7 | 126 |

* Distance along shoot that P. cactorum emerged on culture medium.

** Shoots of MM 104 were heavily colonized by other fungi.

group of fungi was recovered from irrigated Wayne bark in September than from non-irrigated bark in October 1969. In 1970 when apple fruits were used, P. expansum was the only organism recovered from diseased trees in three different orchards.

When P. expansum was inoculated into Beautiful Arcade apple seedlings, it was an aggressive wood invader, suggesting that it may be a primary or secondary parasite which decays the wood followinq or prior to initial infection by P. cactorum. F. oxysporum caused some discoloration of the crowns but did not cause a definite canker. The Nova Scotia isolate of P. cactorum was pathogenic on the seedlings but after 5 months most cankers had healed. Welsh (5) found that high soil moisture is necessary for crown rot development. With increasing temperatures in the greenhouse durinq the spring months, alternate wetting and drying of the upper layers of the soil in the pots may have arrested disease development. This may have also arrested the penetration of P. expansum.

The data on the detached twig inoculations (Table 1) suggest that wood taken in April is more susceptible to invasion by P. cactorum and P. expansum than wood collected in January. With this technique F. oxysporum and P. expansum produced definite sunken cankers and invaded the wood beyond the edges of these cankers. The high susceptibility of MM104 to P. cactorum (3) may in some way be correlated with its susceptibility to secondary invaders.

## Acknowledgments

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