Effects of Month of Inoculation on Severity of Disease Caused by *Phytophthora* spp. in Apple Root Crowns and Excised Shoots

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**ABSTRACT**


Effects of month of inoculation on severity of disease caused by *Phytophthora* spp. in apple rootstock EMLA.106 were studied in excised shoots in vitro and in intact root crowns in an orchard. In both orchard and in vitro assays, 25 successive sets of host tissue were inoculated at monthly intervals with mycelial disks from V8 agar cultures of *Phytophthora* spp. Crown rot severity was measured in orchard trees as area of bark necrosis after 14 days of incubation, and canker severity was measured in excised shoots as length of bark necrosis after 5 days of incubation. In both assays, *P. cactorum* and *P. cambivora* caused very little necrosis in apple inoculated during dormancy (December through February) and growth resumption (March). The amount of necrosis in root crowns and excised shoots was much greater following inoculations in late spring (May). Maximum necrosis and subsequent decline in disease development during incubation periods occurred 1 to 3 months later in orchard tree inoculations (August through October) than in excised shoot inoculations (May through August). Crown rot severity after 2 weeks of incubation was not a reliable predictor of disease severity after 13 months of incubation; relatively mild crown rot caused by *P. cactorum* during and immediately following tree dormancy occasionally continued to develop, resulting in relatively severe crown rot on trees 13 months after inoculation. Under orchard conditions in California, apple rootstock EMLA.106 is apparently susceptible to development of relatively severe crown rot for a longer period of the year than would be expected from short-term indications of susceptibility in excised shoots in vitro.

Additional keywords: collar rot.

Susceptibility of aboveground parts of apple trees to *Phytophthora* spp. is known to fluctuate seasonally (10). Research on the temporal variation has often focused on susceptibility to infection and colonization by *P. cactorum*. Jeffers and Aldwinkle (9) extended an in vitro assay (8) to study seasonal variation in susceptibility of apple to colonization by *P. cactorum*, *P. cambivora*, *P. cryptoegeae*, *P. megasperma*, and *Phytophthora* spp. Sewell and Wilson (17) investigated seasonal effects on development of collar rot caused by *P. cactorum* and *P. syringae*. In shoots and trunks of apple trees, susceptibility to *P. cactorum* and *P. cambivora* was relatively low during tree dormancy and increased to a peak during early spring to summer (9,17). In contrast, development of collar rot caused by *P. syringae* was greatest during tree dormancy (17), and there were two peaks of susceptibility to *P. cryptoegeae*, *P. megasperma*, and *Phytophthora* spp., one during winter and another during summer (9).

Knowledge of seasonal patterns of susceptibility of aerial parts of apple trees to *Phytophthora* spp. has been used to determine optimum periods for evaluating genetic resistance to *Phytophthora* spp. and for applying fungicides to control Phytophthora root and crown rots in apple (1,9). Yet in many regions where apples are grown, including California, the subterranean root crown is the most important locus of infection and colonization by *Phytophthora* spp. Whether seasonal patterns of susceptibility of stems or shoots of apple trees to *Phytophthora* spp. accurately reflect seasonal changes in risk of Phytophthora crown rot development in apple is not known.

We investigated the relationship between temporal susceptibility of excised shoots in vitro and corresponding severity of crown rot development in an orchard to expand basic knowledge on development of Phytophthora crown rots of apple. In addition, we studied temporal effects on crown rot development in relation to tree growth, soil moisture, and temperature variables. A portion of this work was reported previously (4).

**MATERIALS AND METHODS**

**Inoculum.** Single isolates of *P. cactorum* (Lebert & Cohn) J. Schrött., *P. cambivora* (Petri) Buisman (mating type A1), and *P. cryptoegeae* Pethybr. & Lafferty (mating type A1) obtained from apple trees were used for monthly inoculations throughout the study. Stocks of the isolates were maintained in V8 agar slant cultures that were covered with paraffin oil. One set of slant cultures, prepared at the beginning of the study, provided inocula for the first 13 monthly inoculations. A second set of cultures, prepared 1 year later, provided inocula for all subsequent inoculations. In preparation for each monthly inoculation, isolates were transferred from stock cultures to establish fresh V8 agar cultures in petri dishes.

**Root crown inoculations in the orchard.** Effects of month of inoculation on crown rot caused by *Phytophthora* spp. were studied in two adjacent plantings of apple rootstock EMLA.106 near Davis, CA. The plantings were established in two consecutive years. The trees, obtained from a commercial nursery (Trecce Woodburn, OR), were planted as dormant liners at a spacing of 4.6 x 0.9 m. Tree rows were centered on berms about 1 m wide and 5 to 10 cm high above the orchard floor. The plantings were grown under...
standard cultural practices except for monthly experimental inoculations.

Inoculations of successive sets of trees began in the older block of trees in June during the third annual cycle of tree growth after planting. Dates of inoculation were assigned to individual trees in a completely randomized design. On each of the monthly inoculation dates, seven trees were inoculated with *P. cactorum* and seven other trees were inoculated with *P. cambivora*.

To facilitate inoculation, soil at the base of trees was carefully excavated to expose the root crowns. On each exposed crown, a cork borer was used to remove 0.6-cm² disks of bark and expose the cambium tissue. Bark disks were removed from two points on opposite sides of the crown at a depth of 15 cm below the surface of soil on burs. The disks were immediately wrapped in Parafilm to facilitate subsequent measurements of bark moisture in the lab. In each tree, one of the exposed cambium faces was inoculated with a mycelial disk (0.6 cm², mycelial side toward cambium face) from the margin of an actively growing culture of either *P. cactorum* or *P. cambivora*, and the other exposed cambium face was inoculated with a sterile disk of V8 agar as a control treatment. After inoculation, each wound was covered with a 0.6-cm² disk of bark (removed from the tree trunk at approximately 4 cm above the soil line) and a patch of Parafilm (4 cm × 4 cm). Soil was replaced to the original level around the base of each inoculated tree.

On day 14 after inoculation, soil was removed from around root crowns and the extent of crown rot was measured. The surface of the crown bark was scraped with a knife blade (held perpendicular to the bark surface) to reveal margins of healthy (white) and necrotic (reddish brown) tissue. The perimeter of necrotic lesions was traced on clear, threadlike paper. The traces were photocopied, and a planimeter was used to determine area of necrosis enclosed in each trace.

In addition to these primary measurements of crown rot 14 days after inoculation, crown rot severity was assessed again in the oldest block of trees 13 months after inoculation in order to study long-term effects of time of inoculation on colonization by *P. cactorum* and *P. cambivora*. For the later measurement of crown rot, the percentage of the crown circumference that was girdled with decay was determined for each tree.

After the first 13 months of inoculations in the older block of trees, the study was repeated in the younger block. In the second year, inoculations were conducted in the same manner as previously, except that 16 trees were inoculated each month (eight with *P. cactorum* and eight with *P. cambivora*), and crown rot severity was assessed only after the 14-day interval.

**Environmental and biological variables measured in the orchard study.** In the orchard where seasonal development of crown rot was studied, several variables were measured in the trees and soil. Soil temperature was monitored continuously during the successive 14-day periods of incubation at a distance of 15 cm from the tree crown and a depth of 15 cm beneath the soil surface of burs. Soil water matric potential was monitored during the incubation periods at two to three stations in the orchard with tensiometers at 15, 30, and 61 cm beneath the bark surface at a distance of 10 to 30 cm from tree crowns. Rate of shoot extension was determined for monthly time intervals from successive measurements of shoot length for 10 to 20 selected shoots on trees that were selected randomly at the beginning of experiments and remained noninoculated. A qualitative indication of whether cambium cells were actively dividing was obtained each month by noting the ease with which bark disks “slipped” from subtending cambium tissue as trees were prepared for inoculation. The water content of bark removed from crown tissue at inoculation was determined. Thirteen to 29 disks were used for the determinations each month as follows: bark disks were weighed after they were removed from Parafilm (fresh weight), after being hydrated for 1 day in 10 ml of distilled water (turgid weight), and after being dried to constant weight at 90°C (oven-dry weight). The data were used to calculate proportional water content of the bark ([fresh weight minus oven-dry weight] / [oven-dry weight]) and the relative water content of the bark ([fresh weight minus oven-dry weight] / [turgid weight minus oven-dry weight]).

**Excised shoot inoculations.** In experiments that were concurrent with orchard tree inoculations, excised sections of shoots were used to measure temporal changes in susceptibility to *P. cactorum*, *P. cambivora*, and *P. cryptogea* in vitro. Shoot sections were collected randomly from noninoculated trees in the orchard plantings that were used to study temporal effects on crown rot development. Shoot sections were collected and inoculated at the same time as monthly inoculations of root crowns in the orchard (usually within ± 2 days, maximum ± 5 days). Each month, 20-cm shoot sections were excised from the basal 25-cm region of current-season shoot growth, which had matured to the point that attached leaves were fully expanded and wood in the shoots was highly lignified. Additional shoot material was collected to study the effect of shoot age on temporal susceptibility to *P. cactorum*. For this part of the study, 20-cm sections of shoot were selected from 25-cm regions of the previous year’s shoot growth that directly subtended the current-season growth.

In preparation for inoculation, a cork borer was used to remove a 0.2-cm² disk of bark from the middle of each excised shoot. The bark disks were replaced with 0.2-cm² mycelial disks (mycelial side toward cambium face) from margins of colonies of *P. cactorum*, *P. cambivora*, or *P. cryptogea* growing on V8 agar. Sterile disks of V8 agar were used to inoculate controls. Nine to 10 replicate shoots were used for each treatment combination of *Phytophthora* sp. and age class of shoots, and three to four replicate shoots were used for controls.

After inoculation, shoots were incubated (distal ends of shoots upright) in a ventilated 19-L chamber for 5 days at 19 to 23°C and 100% relative humidity. After incubation, length of bark necrosis was measured in each shoot.

**Data analyses.** Data from the orchard and excised shoot studies were subjected to analysis of variance with the SAS general linear models procedure (Version 6; SAS Institute, Cary, NC). Before analysis, the data were corrected to adjust for necrosis at the points of inoculation. The SAS correlation procedure (Version 6) was used to evaluate the means of the environmental and biological variables measured in the orchard study for correlation with mean areas of necrosis.

**RESULTS**

**Root crown inoculations.** In the orchard plantings, necrosis values were affected by a significant interaction of *Phytophthora* sp. with month of inoculation (*P = 0.0001*). No necrosis developed from control inoculations with sterile agar. Root crowns inoculated with *P. cactorum* and *P. cambivora* during tree dormancy (December through February) and growth resumption (March) showed very little necrosis after 2 weeks of incubation (Fig. 1A). However, development of necrosis increased in late spring (May). In summer months (June through October), necrosis remained relatively high, except during August and October in the first year of study, when relatively little crown rot developed from inoculation with either *P. cactorum* or *P. cambivora* (Fig. 1A). In fall of both years of the study, *P. cactorum* and *P. cambivora* caused significant necrosis through the period of incubation in early November. During several periods in summer and fall (Julian days 244 and 302 in year 1, 212 and 275 in year 2, and 151 in year 3), *P. cactorum* caused significant necrosis (*P = 0.05*) more necrosis than did *P. cambivora* (Fig. 1A).

*Phytophthora* spp. were isolated from necrotic crown tissue after several 2-week periods of incubation. *P. cactorum* and *P. cambivora* were consistently recovered from the small areas of necrotic tissue that developed after inoculation of dormant trees as
well as from larger areas of decay that developed after inoculation of trees in spring, summer, or fall.

Thirteen months after dates of inoculation, most cankers caused by *P. cambivora* had ceased to develop and nearly healed, whereas many crown lesions initiated by *P. cactorum* had continued to expand (Fig. 2). Interaction of *Phytophthora* sp. with date of inoculation was statistically significant (*P = 0.0001*). The expansion of bark cankers caused by *P. cactorum* was particularly evident in tree crowns inoculated during December through February, because bark necrosis in those trees was relatively limited after the initial 2 weeks of incubation (Figs. 1A and 2).

**Environmental and biological variables.** In the orchard study, the amount of necrosis that developed within 2 weeks after inoculation with *P. cactorum* or *P. cambivora* was positively correlated with several environmental and plant growth variables, including mean proportional water content of bark samples, mean growth rate of shoots in the month before inoculation, and mean soil temperature at a depth of 15 cm (*P = 0.0001* to 0.007) (Table 1 and Fig. 1A to C). In contrast, necrosis was not significantly corre-

![Graphs showing variables measured in apple orchard study.](image)

**Fig. 1.** Variables measured in apple orchard study. A. Severity of necrosis caused by *Phytophthora* spp. in apple root crowns as a function of date of inoculation. Successive sets of trees were inoculated, and disease was assessed after 2 weeks of incubation. Vertical bars delimit standard error of means. B. Water content of bark samples on date of inoculation, shoot growth rate (plotted according to change in shoot length on noninoculated trees during nominal months of incubation), and periods of apparent cambium division (delimitated according to qualitative tendency of bark disks to "slip" from subtending cambium tissue on dates of inoculation). Vertical bars delimit standard error of means. C. Soil water potential and soil temperature. Vertical bars delimit standard error of matric potential means and maximum and minimum temperatures during incubation periods. Water matric potential was measured 10 to 30 cm from tree trunks at a soil depth of 61 cm. Temperature was measured 15 cm from tree trunks at a soil depth of 15 cm.
of both years of study. Following peaks of susceptibility in May, mean lengths of cankers caused by *P. cactorum* and *P. cambivora* were relatively high but generally declined over summer months (Fig. 3A). However, cankers caused by *P. cactorum* and *P. cambivora* in August of the first year were about three to four times as long as corresponding cankers in August of the second year. Susceptibility of excised shoots to both *Phytophthora* spp. declined in early fall, and canker development became negligible by October and November (Fig. 3A). The mean monthly canker lengths in excised shoots were not significantly correlated with the mean necrosis area in root crowns for either *P. cactorum* or *P. cambivora* (*P* = 0.61 to 0.14).

In contrast to *P. cactorum* and *P. cambivora*, *P. cryptogea* caused the most severe cankers in vitro during months of dormancy (January to March) (Fig. 3A). Susceptibility to *P. cryptogea* was variable during the spring, summer, and fall months.

**Effect of shoot age.** The general seasonal pattern of canker development was similar in “current-season” shoots and “year-old” shoots (Fig. 3B). Both classes of shoots developed negligible amounts of necrosis during tree dormancy but increased in susceptibility to *P. cactorum* during the late spring and summer months (May to August). However, susceptibility peaked and declined about 1 month later in “year-old” shoots than in “current-season” shoots (Fig. 3B). The statistical interaction of shoot age with date of inoculation was significant (*P* = 0.0001).

**DISCUSSION**

The results of in vitro assays of susceptibility in apple shoots to *P. cactorum*, *P. cambivora*, and *P. cryptogea* agree in most respects with results obtained previously with similar methods of inoculation (1,5,6,7,9,11,12,16,17,18). Previous studies detected a lack of susceptibility to *P. cactorum* and *P. cambivora* in excised apple shoots during shoot dormancy and a pronounced increase in susceptibility after shoot growth commenced (1,5,6,9,12,16,17,18). When Jeffers and Aldwinkle (9) inoculated excised shoots of MM.106 and MM.111 rootstocks in New York State with *P. cryptogea*, they observed two peaks of susceptibility, one during summer and another during winter. In the present study, no consistent peak of susceptibility to *P. cryptogea* was observed in the summer, but in two successive years a peak of susceptibility to the fungus occurred in January (Fig. 3A). The lack of a second peak in susceptibility to *P. cryptogea* in summer in our study may reflect the influence of environmental differences between Davis, CA, and Geneva, NY, or differences between fungal isolates or procedural details.

Previous work with *P. citricola* on walnut (15) and *P. citrophthora* and *P. parasitica* on citrus (13,14) rootstocks revealed, as did results of the present study, that seasonal patterns of susceptibility to *Phytophthora* spp. can be affected by methods of inoculation, type of host tissue inoculated, and the *Phytophthora* sp. used for inoculation. In the citrus and walnut studies, susceptibility to colonization by the *Phytophthora* spp. used in inoculations was generally low during winter months and comparatively high at other times, which corresponds broadly with results of apple studies with *P. cactorum* and *P. cambivora* (1,5,6,9,11,12,16,17,18).

The excised shoot procedure we used did not give dependable predictions of the susceptibility of root crowns to colonization by *P. cactorum* and *P. cambivora* in orchards. In addition, excised shoots were often more susceptible to *P. cambivora* than to *P. cactorum*, whereas the opposite was true in root crowns of orchard trees. Differences between results of the orchard and in vitro assays cannot be attributed to any particular factor(s), because the two assays differed not only in the plant parts that were inoculated, but also in environmental conditions.

The fact that *P. cactorum* often caused much more crown rot than did *P. cambivora* during the 2-week and 13-month periods of incubation may indicate that *P. cactorum* is more virulent than *P. cambivora* in root crown tissue of apple trees. Conversely, greenhouse experiments (3) indicate that *P. cambivora* is more virulent than *P. cactorum* in small roots. The apparent affinities of *P. cactorum* for attacking root crowns and of *P. cambivora* for attacking supporting roots are consistent with field observations (S. M. Miretich and G. T. Browne, unpublished).

Our results suggest that it is wise to place undue emphasis on short-term measurements of susceptibility to *Phytophthora* spp. in apple. In this study, severity of disease assessed 5 days after inoculation of excised shoots and 14 days after inoculation of root crowns did not reflect the long-term consequences of colonization of root crowns by *P. cactorum* or *P. cambivora*. For example, although *P. cactorum* caused negligible disease in apple tree crowns within 2-week periods of incubation in August, October, and December through February of the first year of study, the initial exposure to the fungus during these periods led ultimately to development of crown rot in trees within 13 months after inoculation.

Many of the specific factors that govern seasonal changes in susceptibility to *Phytophthora* spp. remain unknown. Because of the direct method of inoculation, our orchard study bypassed events that preceded and accompanied natural host penetration. Nevertheless, it appears that activities of the host related to growth can be associated with increases in severity of disease caused by *P. cactorum* and *P. cambivora*. For example, necrosis caused by *P. cactorum* and *P. cambivora* in root crowns was positively correlated with rate of apical shoot growth. Likewise, crown root development within 2 weeks of inoculation was extensive when there was qualitative evidence of cell division in cambium of root crowns but was negligible in the apparent absence of cell division in the cambium.

![Crown rot girdling (%)](image)

**Fig. 2.** Severity of crown rot caused by *Phytophthora* spp. 13 months after inoculation of orchard trees, plotted against date of inoculation. Vertical bars delimit standard error of means.

**TABLE 1.** Correlation matrix for variables measured in the orchard study on crown rot of apple caused by two species of *Phytophthora*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient of correlation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>with area of necrosis caused by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean relative water content of bark</td>
<td>0.13</td>
<td>0.31</td>
</tr>
<tr>
<td>Mean proportional water content of bark</td>
<td>0.56**</td>
<td>0.73**</td>
</tr>
<tr>
<td>Mean growth rate of shoots during month before inoculation</td>
<td>0.64**</td>
<td>0.61**</td>
</tr>
<tr>
<td>Mean growth rate of shoots during month after inoculation</td>
<td>0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean soil temperature at 15-cm depth</td>
<td>0.53**</td>
<td>0.68**</td>
</tr>
<tr>
<td>Mean soil water matric potential at 61-cm depth</td>
<td>0.24</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Two asterisks indicate statistical significance at *P* < 0.01.
Our results highlight the importance in disease prevention of careful soil water management to avoid periods of saturation that favor infection (2). Given conventional orchard practices, it appears that once P. cactorum or P. cambivora has initiated colonization of apple crown tissue, subsequent development of crown rot is not influenced greatly by relative water content of bark of root crowns or water matric potential in surrounding soil. Neither relative water content of bark (which remained relatively constant during the study) nor soil water matric potential at 61-cm depth was significantly correlated with mean severity of crown rot in the orchard. In addition, drying of soil adjacent to tree crown at a depth of 15 cm to less than -0.8 bar during summer months did not prevent development of severe crown rot caused by P. cactorum or P. cambivora. After infection occurs, it may be impossible to use soil water management within acceptable horticultural limits to influence subsequent colonization by P. cactorum or P. cambivora.

Our results indicate that colonization of apple crown tissue, once initiated, may lead to development of severe crown rot in apple rootstock EMLA 106 during a relatively extended period of the year. This extended period of risk emphasizes the desirability of rootstocks, cultural practices, and fungicides that offer continuous protection from infection and colonization by Phytophthora spp.

LITERATURE CITED