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Incidence of *Phytophthora cactorum* Crown and Root Rot on Seven Apple Rootstocks Artificially Infected in the Orchard

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Abstract

Seven apple rootstocks budded with 'Jonagold' scion were inoculated with *Phytophthora cactorum* annually for four years and evaluated for susceptibility to this pathogen in orchard plots at the Pacific Agri-Food Research Centre at the end of the fourth growing season. No rootstock was observed to be completely resistant to *P. cactorum* crown and root rot. The rootstock O. 3 was significantly less susceptible to *P. cactorum* infection compared with the MM.106 EMLA rootstock, which was the most susceptible. The rootstocks B. 9, J. 9, P. 2, M. 9 EMLA, and M. 26 EMLA were least susceptible to crown and root rot. This study indicated that it was possible to assess resistance to *P. cactorum* in the orchard.

Introduction

In the Okanagan, Similkameen, and Kootenay valleys of British Columbia, crown and root rot of apple trees *Malus domestica* Borkh.) is primarily caused by *Phytophthora cactorum* (Leb & Cohn) Schroet.(16, 22). *P. cambivora* (Petri) Buism has been isolated from roots of apple in the Okanagan Valley but it is not as prevalent as *P. cactorum*. Other species including *P. cryptogea* Pethy & Laff.; P. *citricola*, Sawada; *P. syringae*, (Kleb.) Kleb.; *P. megasperma*, Dreschler; P. *drechsleri*, Tucker; *P. cinnamomi*, Rands; *P. parasitica*, Dastur; *P. citrophthora*, Smith & Smith (8, 10, 15, 17, 21) have been found to be associated with crown and root rot in other parts of the world. However, they were not found in the Okanagan and Similkameen Valleys (16).

Rootstocks of apple vary in their resistance to *P. cactorum* (16). Among the rootstocks used in British Columbia, M.9 and M.4 are considered resistant to *P. cactorum* crown and root rot, whereas MM.104 and MM.106 rootstocks are highly susceptible. M.26 is less resistant to *P. cactorum* than M.4 (25). M.9 and M.26 remain the most widely planted rootstocks in British Columbia, wheras MM.106 is no longer planted (18).

Inoculation of excised shoots or roots and variations of this technique have been developed to screen rootstocks and scions for crown rot resistance (1, 2, 4, 9, 14, 19, 23, 24, 25). These methods are simple, convenient and reproducible, but the results obtained by these methods do not always reflect relative field resistance especially of rootstocks. The results of cut-shoot tests showed that scion and rootstock resistance as measured by cut-shoot test varied greatly with the stage of growth (5, 13, 23). Gates and Millikan (5) found that cuttings were highly susceptible when they were collected during blossom time. Resistance of cuttings were also highly dependent on the position of the shoot on the tree (12).

Polish (P.) 2, Ottawa (O.) 3, Jork (J.) 9, and Budagosky (B.) 9 are hardy, dwarfing rootstocks that have been selected for cold sites (18). These rootstocks have been evaluated for resistance to *P. cactorum* by cut shoot artificial inoculation but have not been evaluated in the orchard (23). The purpose of this study was to induce crown and root rot symptoms under orchard conditions and to determine their susceptibility of four hardy rootstocks with M.9 EMLA, M.26 EMLA, and MM.106 EMLA as standards. These rootstocks

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were selected as standards because their relative resistance is known (16). M.9 EMLA is classed as resistant rootstock. M.26 EMLA is moderately susceptible and MM.106 EMLA is very susceptible rootstock to *P. cactorum* crown rot under orchard conditions.

Materials and Methods

The orchard trial was conducted at the Pacific Agri-Food Research Centre at Summerland in British Columbia (B.C). The soil in the orchard trial area was sandy loam with a pH of 6.9. All virus free rootstocks were obtained from the Plant Quarantine Station in Sydney, B. C. and were multiplied in a nursery at the Pacific Agri-Food Rresearch Centre, Summerland, B. C. Two-year-old trees budded with 'Jonagold' were planted. Rootstocks were arranged in a complete randomised block design with 21 replications of one tree each. Tree spacing was 3.5 x 1.5m.

Soil around each tree was infested with *P. cactorum* annually for 4 years in mid-June. The soil was removed from the crown region to a depth of about 4 cm and replaced immediately after application of the fungal pathogen. To obtain spores of *P. cactorum* for soil infestation, isolate $PH0_3$was grown on corn meal agar (CMA) for 1 wk at 18 C. Clarified V-8 broth was prepared from V-8 juice (Campbell Soup Co, Toronto, ON). Calcium carbonate ($CaCO_3$, 7.9 g) was added to 538 g of V-8 juice and centrifuged for 20 min at 4000 rpm. The supernatant was diluted with distilled water in the proportion 1:4 and then autoclaved (121 C, 20 min). Mycelium from cultures was macerated along with agar to a fine consistency in a tissue culture grinder with 5 ml of clarified V-8 broth under sterile conditions. The ground material was poured into culture bottles containing 100 ml V-8 broth and mixed well and 5 ml aliquots were pipetted into 60 x 15 mm petri plates. These cultures were incubated for 2 days at 25 C and then at 18 C for 3-4 wk in darkness. Suspensions of sporangia were obtained by adding mycelial mats to 100 ml sterile water and blending in a sterile blender at 10-sec. intervals for 2 min. The stocks were diluted to obtain approximately 5300 colony forming units (CFU) per ml. To infest soil, soil was removed from the crown region (4 cm from the tree trunk) to a depth of about 6 cm. Ten ml of stock suspension of *P. cactorum* diluted to 50 ml with sterile water was poured evenly around the base of the tree, and the soil was replaced immediately. The trees were irrigated with drip emiters located at every 20 cm for 4 hr immediately after the soil infestation with *P. cactorum*. During the growing season the test plot was irrigated for 4 hr at 2 day intervals. Weeds, insects, and foliar diseases were controlled by standard orchard practices. The trial was terminated in the fall of the fourth season, after inoculating 4 times. Tree tops were cut off with a chain saw, and the stumps were removed carefully. Roots (0 - 30 cm depth) were evaluated visually for root rot damage immediately after removal from soil. The evaluation was based on the percent (0 - 100%) of the bark and root decay in twenty randomly selected bark and root pieces (10 mm long and 1 mm diameter) from each tree in each rootstock.

The presence or absence of *P. cactorum* in the bark and roots of infected trees was confirmed following the method used by Matheron et al (15), on ten plates per sample, using a selective medium containing corn meal agar amended with 5 mg of pimaricin, 300 mg vancomycin hydrochloride, and 25 mg of pentachloronitro-benzene per litre. The plates were examined daily for 3-5 days for growth of *P. cactorum*. Colonies typical of *P. cactorum* were examined under a microscope to confirm infection by the pathogen.

All data were analyzed by General Linear Model (GLM) procedures (SAS Institute, Cary, NC) . Data were analyzed for statistical significance using an arc sine transformation for percentages. Duncan's multiple range test was used to compare rootstocks after an ANOVA showed significant differences among means.

Phytophthora cactorum was isolated out of bark and root samples from all trees showing typical symptoms of crown and

root rot, but was not isolated from trees not showing symptoms. The range of infection was from 0 % to 100 % in individual trees.

Results and Discussion

McIntosh (16) concluded that a reliable method of evaluating the resistance of rootstocks to crown rot was required. An essential prerequisite for this is the ability to induce the disease experimentally in susceptible rootstock types under field conditions. Reported successes in experimentally inducing crown rot in clonal rootstocks have been few (20). Crown rot has proved to be more difficult to reproduce consistently in rootstocks of grafted trees under orchard conditions (6). We have been able to produce crown and root rot under orchard conditions by inoculating the soil with spores of *P. cactorum* every June for 4 years and irrigating the soil immediately after inoculation.

The General Linear Model procedure for the percentage of infection was significant at P = 0.05. Infection by *P. cactorum* started after two years from first inoculation in a few trees. Significant differences in susceptibility to *P. cactorum* were observed among the rootstocks (Table 1). As expected, MM. 106 was highly susceptible (45.6%) followed by O. 3 (25.7%); there was a significant difference between them. The rootstock B. 9 was least affected (7.3%) by *P. cactorum* crown and root rot infection among all the rootstocks tested. Browne and Mircetich (3) also found that B.9 and M.9 EMLA were higly resistant to *P. cactorum* in artificially infested soil under controlled conditions. No significant differences in *P. cactorum* crown and root rot infection were observed between B.9, J.9, P.2, M. 9 EMLA, and M.26 EMLA.

M.26 has been considered moderately to very susceptible (11). In the present study, significant difference was observed between MM.106 and M.26 to *P. cactorum* infection. These field observations are similar to *in vitro* studies by Utkhede and Quamme (23) and greenhouse studies by Wilcox (26) and field studies by Utkhede and Smith (25). Based on cut-shoot tests Wilcox (26) showed that M.26 was significantly less susceptible than MM.106. In a pot experiment, MM.106 was 100% susceptible to *P. cactorum*, while M.26 was least susceptible (20% infection) after 72h flooding (26). Under orchard conditions, Utkhede and Smith (25) observed that there was a significant difference in *P. cactorum* infection between MM.106 and M.26 obtained from the same nursery (Budwood Orchard Nursery). However in the same field trial, no significant difference in *P. cactorum* infection was observed between MM.106 obtained from Budwood Orchard Nursery and M.26 obtained from Traas Nursery (25). Lack of agreement of crown rot tests with orchard performance has been reported before (10) and appears to be a common problem with crown rot assays.

Utkhede and Smith (25) observed that two rootstocks (M.26 and MM.106) from two sources (British Columbia Fruit Growers Association Budwood Orchard, Summerland, and Traas Nursery, Langley, British Columbia) showed no significant differences in their susceptibility to *P. cactorum* infection in the final observations (1991 and 1992). However, MM.106 from Budwood Orchard Nursery was more susceptible to *P. cactorum* compared with the MM.106 from Traas Nursery from 1984 to 1990. Based on these observations they suggested that resistance of rootstocks to *P. cactorum* may vary from year to year for some rootstocks.

The present field study has shown that B.9, J.9, P.2, M.9 EMLA, and M.26 EMLA were significantly more tolerant to *P. cactorum* infection compared with MM.106 and O.3. Previously, results of a cut shoot test indicated that J.9, M.9 EMLA, P.2 and O.3 were more resistant than B.9 and all were more resistant than MM.106 (23). Barritt et al. (1) found that B.9 and P.2 were more resistant than MM. 106 in two of two tests and M. 9 EMLA, M. 26 and O. 3 were more resistant than MM.106 in one of two tests. Lemoine and Gaudin (13) found O. 3, M.9 EMLA, B.9 and M.26 to be more resistant than MM.106. Resistant readings from the cut

Table 1. *Phytophthora cactorum* crown and root rot infection induced in seven rootstocks by artificial inoculation under orchard conditions compared to the lesion length in inoculated shoots. (23).

Rootstock	Lesion length in inoculated shoots (April measurement) (mm)	Crown and root rot infection (%)*
B. 9	33.0 b**	7.3 a
J. 9	15.8 a	9.0 a
P. 2	19.8 a	9.3 a
M. 9 EMLA	18.5 a	9.8 a
M. 26	35.5 b	10.7 a
O. 3	25.0 a	25.7 b
MM. 106	45.0c	45.6 c
S. E.	3.5	5.1

*Percent infection was based on the decay in twenty randomly selected bark and root pieces from each tree in each rootstock.

**Means followed by the same letter within a column are not significantly (0.05) different according to Duncan's multiple range test.

shoot test have been consistent with field readings except for O.3, M.26 and possibly B.9. Ottawa3 had a lower rating in the field test than in cut shoot tests. In pot studies, Browne and Mircetich (3) reported that M.9 EMLA and B.9 were highly resistant whereas MM. 106 EMLA was susceptible. The lower level of field resistance of O.3 is consistent with the observation by Wilcox (26) when growing plants were inoculated with *P. cactorum* in pots. In this study O.3 rated lower resistance to *P. cactorum* than M.26 but higher than MM.106. However, O.3 was among the most resistant of the rootstocks to *P. cambivora* and *P. megasperma*. The measurement of *P. cactorum* resistance on B.9 in the orchard test was higher (equal to M.9 EMLA) compared to that in the cut shoot test (equal to M.26). However, readings on B.9 in the cut shoot tests conducted by Barritt et al. (1) and Lemoine and Gaudin (13) was lower. M.26 was rated higher in our orchard test than it did in a cut shoot test (lower than M.9 EMLA) conducted by Utkhede and Quamme (23) but the rank was the same (between M.9 EMLA and MM.106). Barritt et al. (1) and Lemoine and Gaudin (13) also found that the resistance of M.26 ranks between M.9 EMLA and MM.106. Cut-shoot inoculations during the growing season showed relative ranking of several rootstocks but failed to demonstrate the comparative susceptibility of MM.104 and MM.106 (6). Jeffers and Aldwinckle (7) similarly found little difference between MM.104 and MM.106 in their response to cut-shoot inoculations with *P. cactorum*.

Orchard tests appears to be a more reliable method than the cut shoot test for evaluating rootstocks for their resistance to *P. cactorum*. The results of this study highlight the importance of the orchard study to determine the resistance of apple rootstocks. However, the results of the orchard study could be variable with different species of *Phytophthora*. The method of inoculating cut woody shoots has been used in various forms by many workers to measure the resistance of apple trees to *P. cactorum*, but this technique has generally proved to be a more useful indicator of scion tolerance to collar rot than of rootstock resistance to crown rot (16, 4).

Conclusions

This study has shown that no rootstock was completely resistant to *P. cactorum* crown and root rot. The rootstock O. 3 was less susceptible to *P. cactorum* infection compared with the MM.106 EMLA rootstock, which was the most susceptible. The rootstocks B. 9, J. 9, P. 2, M.9 EMLA, and M.26 EMLA were least susceptible to crown and root rot. It was shown that it is possibile to assess resistance to *P. cactorum* in the field. This study indicated that the variation in resistance to *P. cactorum* infection exists and may be explored to develop crown and root rot resistance in apple rootstocks.

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