Relative Resistance of Eighteen Selections of *Malus* spp. to Three Species of *Phytophthora*

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**ABSTRACT**


Eighteen selections from 13 species and hybrids of *Malus* (*M. baccata*, *M. brenipes*, *M. coronaria*, *M. domestica*, *M. fisa*, *M. halliana*, *M. Ioenis*, *M. × magdeburgensis*, *M. mandshurica*, *M. × playcarpa*, *M. prunifolia*, *M. × sargentii*, and *Malus* sp.) were evaluated in a greenhouse for resistance to root and crown rots caused by *Phytophthora cactorum*, *P. cambivora*, and *P. cryptogeae*. Seven- to nine-week-old seedlings were transplanted into noninfested soil or soil artificially infested with *P. cactorum*, *P. cryptogeae*, or *P. cambivora*. The soil was flooded once every 2 wk for 48 h, and plants were evaluated for severity of disease 3 mo after transplanting. Seedlings of domestic apple (*M. domestica*, used as a standard for comparison in this study) were among the most susceptible of the *Malus* spp. to crown and root rot caused by *P. cactorum*, *P. cryptogeae*, and *P. cambivora*. The relative resistance of other selections of *Malus* spp. varied according to species of *Phytophthora* and whether crown or root rot variables were used to assess resistance. Among 10 selections of *Malus* spp. that were evaluated along with two selections of *M. domestica* for resistance to *P. cactorum*, *P. cryptogeae*, and *P. cambivora*, six selections were relatively resistant compared to *M. domestica* to root and crown rots caused by *P. cactorum*, but only *M. halliana*, *M. × magdeburgensis*, and *M. × sargentii* were relatively resistant to root and crown rot caused by all three fungi. Species of *Malus* can vary greatly in resistance to species of *Phytophthora*, and, among *Malus* spp., assessments of relative resistance to *P. cactorum* are not necessarily extendible to other *Phytophthora* spp.

Additional keyword: rootstocks.

The diversity among and within species of *Malus* is a valuable resource for genetic resistance to various pests and diseases. Genetic resistance to *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. has been expressly sought among *Malus* spp. in breeding programs for apple rootstocks (3,8,10,24), and results of controlled studies indicate that germ plasm of *Malus* can vary greatly in resistance to this *Phytophthora* sp. Detection, inheritance, and utilization of genetic resistance to *P. cactorum* among selections of *Malus* spp. have been investigated (1,3,8,10,15,16,21,22,24).

Although much has been learned about genetic resistance to *Phytophthora* root and crown rots in apple, etiological and procedural complications remain. The disease can be caused by a complex of *Phytophthora* spp., but evaluation of genetic resistance among *Malus* spp. to *Phytophthora* root and crown rots has been limited to studies with *P. cactorum* (12,17). Whether resistance of *Malus* spp. to *P. cactorum* is associated with resistance to other species of *Phytophthora* that commonly cause root and crown rots of apple is largely unknown (12), and this is an important concern for apple rootstock breeders. Additionally, large numbers of individuals often must be tested at one time in rootstock breeding programs (8); for this reason, workers have often used mass inoculations of small and very young seedlings (i.e., 10 days old) for evaluations of resistance to *P. cactorum*. Expression of resistance in apple selections at this early seedling stage may not necessarily resemble the resistance of selections at later stages of development.

This study was undertaken to expand knowledge of relative resistance of different *Malus* spp. to *Phytophthora* root and crown rots. Most of the *Malus* spp. we utilized do not serve as apple rootstocks, but they are of interest to plant breeders as sources of genetic resistance to pests and diseases. We investigated relative resistance of *Malus* spp. to *P. cactorum*, *P. cryptogeae*, and *P. cambivora*, all of which are implicated as important root and crown pathogens of apple (12). Seedlings were intentionally evaluated for resistance at an older age in this study than in previous studies by other workers. A portion of this research was reported previously (7).

**MATERIALS AND METHODS**

Germ plasm. Seeds of the *Malus* spp. evaluated for resistance to *Phytophthora* spp. were obtained from three sources (Table I). One lot of domestic apple (*M. domestica* Borkh.) and one lot of *M. × sargentii* Rehd. were obtained from Schumacher Co. (Sandwich, MA). Another lot of domestic apple seed was obtained from Morton Nursery (Yakima, WA). All remaining seed lots of *Malus* spp. tested were supplied by the USDA-ARS National Clonal Germplasm Repository at Geneva, NY. The repository seeds were produced by open pollination in an orchard collection of *Malus* spp.

The nomenclature used for *Malus* spp. in this report conforms to the nomenclature used by the Germplasm Resources Information Network (GRIN, personal communication, J. H. Wiersema, USDA-ARS). Selections listed previously (7) as *M. bracteata* Rehd., *M. glabrata* Rehd., and *M. glaucescens* Rehd. are all considered to be synonyms of *M. coronaria* (L.) Mill. In addition, selections previously listed (7) as *M. baccata* var. *mandshurica* (Maxim.) Schneid., *M. rockii* Rehd., and *M. tuerbi* are listed here in conformity to GRIN as *M. mandshurica* (Maxim.) V. Komarov, *M. baccata* var. *himalaica* (Maxim.) Schneid., and *Malus* spp., respectively.

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Production of seedlings. In preparation for stratification, the seeds were moistened fully by soaking in clean tap water for 3–4 days at 20–24°C. After hydration, the seeds were placed in polyethylene bags with coarse horticultural vermiculite that had been moistened with 200–250 ml of water per liter of dry vermiculite. Care was taken not to saturate the medium with water. The seeds were held in moist vermiculite for 3 mo at 5–6°C. After stratification, seeds were planted in 175-ml pots (one seedling per pot) that contained 100 ml of U.C. mix soil (4). Most seeds exhibited radical emergence at the time of planting. The seedlings were grown in a greenhouse and fertilized weekly with a complete Hoagland solution in which all sources of nitrogen were nitrates (11).

Isolates of Phytophthora and production of inoculum. Isolates of P. cactorum, Phytophthora cambivora (Petri) Buisman, and Phytophthora cryptogea Pethybr. & Lafferty were used in three experiments. In the first two experiments, P. cactorum included a mixture of two isolates from apple, P. cambivora included a mixture of two isolates from apple, and P. cryptogea included a single isolate from apple. In a third experiment, P. cactorum included a mixture of four isolates from apple, P. cambivora included a mixture of three isolates from apple and one isolate from a surface source of irrigation water, and P. cryptogea included one isolate from apple. All isolates were obtained from locations in California.

Inocula were grown on a vermiculite-based medium (5). In each experiment, inocula of the individual isolates of Phytophthora spp. were grown in separate containers. At the time of soil infestation, inocula of isolates within a Phytophthora sp. were combined and mixed thoroughly by hand; each isolate was represented in equal proportion in the mixtures. Sterile vermiculite-based medium was used for nonincubated controls.

Evaluation of resistance to Phytophthora spp. Experiments were conducted in a greenhouse where incandescent lighting maintained a 15-h photoperiod. At 7–9 wk after planting, the seedlings (with soil around the roots) were transplanted into 1-L pots with 0.9 L of U.C. mix soil that was either noninfested or artificially infested with P. cactorum, P. cambivora, or P. cryptogea (5). The inocula and sterile vermiculite-based medium for noninfested controls were added at a rate of 30 ml per liter of soil and mixed thoroughly by hand to achieve uniform dispersal in the soil. After seedlings were transplanted, the soil was fertilized with Hoagland solution (11) every 4–7 days.

Ten days after transplanting and once every 2 wk thereafter, the soil in each pot was flooded for 48 h to favor infective activity of the Phytophthora spp. In a previous study, 48-h flooding periods stimulated production and release of zoospores of P. cactorum, P. cambivora, and P. cryptogea and resulted in root and crown rot of apple seedlings (5). To facilitate flooding, the pots were placed in bowls that were slightly larger than the pots. Water then was added to maintain the surface of flood water at 0.5–1 cm above the surface of soil in the pots. Forty-eight hours after initiation of flooding, the pots were removed from the bowls and allowed to drain. Between episodes of flooding, the soil in each pot was watered every 1–2 days as needed and allowed to drain freely. During experiments, soil temperature ranged from 17 to 30°C.

Three months after transplanting, seedlings were evaluated for severity of disease. The percentage of each seedling root system that appeared rotted was estimated visually (5). In addition, severity of crown rot (i.e., the extent of decay at the stem base and main roots) was assessed for each seedling as the percentage of the crown circumference that was rotted (visual estimate) and as the measured vertical length of crown lesions.

A split plot design was used in each of three experiments (14). Species of Phytophthora were randomized among main plots that were distributed in complete blocks, and individual seedlings (subplots) of Malus spp. were randomized within main plots. In the first two experiments, four to six replicate seedlings (one per pot) were used per combination of Phytophthora sp. and selection of Malus; 18 selections of Malus spp. were evaluated with P. cactorum and P. cryptogea, and 12 selections were evaluated with P. cambivora. In the third experiment, six replicate seedlings (one per pot) were used for each treatment combination, and four selections of Malus spp. were evaluated.

Data were subjected to analysis of variance with the SAS general linear models procedure (13) after transformations. In all three experiments, the arc sine square root transformation was applied to the variables percent root rot and percentage of crown circumference rotted, whereas the square root transformation was applied to crown root length data. For experiments one and two, F tests indicated that error variances were sufficiently stable between the experiments for a combined analysis of variance. In these two experiments, degrees of freedom were combined to calculate mean squares that were appropriately corrected for responses of zero for root and crown rot variables. Means and confidence intervals from transformed data were detransformed (14) before presentation herein.

RESULTS

In the first two experiments (Fig. 1), analysis of variance of the data from the 12 selections of Malus evaluated for resistance to P. cactorum, P. cambivora, and P. cryptogea revealed a consistent and statistically significant interaction of Phytophthora sp. x selection of Malus sp. (P = 0.020, 0.001, 0.0001 for percentage of crown girdling, length of crown rot, and percentage of root rot, respectively). Treatment means from combinations of Malus sp. and Phytophthora sp. did not vary significantly between experiments, i.e., there was no significant interaction of experiment x Phytophthora sp. x selection of Malus sp. (P = 0.51, 0.80, and 0.61 for percentage of crown girdling, length of crown rot, and percentage of root rot, respectively). Likewise, with the complete set of 18 selections of Malus spp. evaluated against P. cactorum and P. cryptogea, a significant interaction occurred between Phytophthora sp. x selection of Malus sp. (P = 0.008, 0.002, and 0.002 for percentage of crown girdling, length of crown rot, and percentage of root rot, respectively). Results of the analyzes of variance were used as a basis for presentation of Phytophthora sp. x selection of Malus sp. means from combined experiments one and two. Plants grown in noninfested soil (controls) developed negligible root rot (means 1–5%, data not shown) and no crown rot, so the data from the control plants were not subjected to analysis of variance or separation of means.

![Table 1: Selections and sources of Malus spp. evaluated for resistance to Phytophthora spp.](image-url)
In the first two experiments, both selections of *M. domestica* were among the most susceptible of the *Malus* spp. to crown and root rot caused by *P. cactorum*, *P. cambivora*, and *P. cryptogea* (Fig. 1A–C). Relative to *M. domestica*, resistance of the other selections of *Malus* spp. depended upon which species of *Phytophthora* was present, and, to some extent, upon whether root or crown rot variables were used to assess resistance.

In soil infested with *P. cactorum*, selections of *M. domestica* developed moderate crown rot (mean percent girdling 41–49%, mean length 14 mm, Fig. 1A and B), and *M. baccata* (L.) Borkh.

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**Fig. 1.** Relative resistance of 18 selections of *Malus* spp. to *Phytophthora cactorum*, *P. cambivora*, and *P. cryptogea* as assessed by A, mean severity of crown rot girdling; B, crown rot length; and C, percentage of root rot. Seedlings of *Malus* spp. were grown for 3 mo in noninfested soil or soil infested with one of the three *Phytophthora* spp. Means are from two experiments, *n* = 8–12. In noninfested controls, no crown rot occurred and mean root rot ranged from 1–5% (data not shown). For each variable used to assess resistance, statistical interaction of *Phytophthora* sp. × *Malus* spp. was statistically significant (*P* = 0.02–0.0001, data from controls excluded). NT designations indicate combinations not tested, and vertical bars delimit confidence intervals (95%) for comparison of means within inoculum treatments.
and *M. mandshurica* developed slightly less crown rot (mean girdling 30%, mean length 5–6 mm, Fig. 1A and B). All other selections developed moderately little crown rot (mean girdling 3–8%, mean length 0–1 mm Fig. 1A and B). *P. cactorum* caused moderate root rot in selections of domestic seedling (means 32–44%, Fig. 1C); slightly less root rot in *M. baccata*, *M. mandshurica*, and selections of *M. coronaria* (means 12–29%, Fig. 1C); and comparatively less root rot in the other selections of *Malus* spp. (means 1–7%, Fig. 1C).

Resistance to *P. cactorum* was accompanied by susceptibility to *P. cryptogaea* in several selections of *Malus* spp. For example, *M. fusca* (Raft.) C. Schneid., *M. × platycarpa* Rehd., and selections L4, L18, and L19 of *M. coronaria*, which were resistant to crown rot caused by *P. cactorum*, sustained moderate to severe levels of crown rot in soil infested with *P. cryptogaea* (Fig. 1A and B). Similarly, *M. fusca*, *M. ioensis* (A. Wood) Britton, *M. × platycarpa*, and selections L4, L18, and L19 of *M. coronaria*, which were all relatively resistant to root rot caused by *P. cactorum*, sustained relatively high levels of root rot in soil infested with *P. cryptogaea* (Fig. 1C). In contrast, *P. cryptogaea* caused relatively little crown or root rot in most other selections of *Malus* spp. that were relatively resistant to root and crown rot caused by *P. cactorum* (Fig. 1A-C).

In soil infested with *P. cambivora*, *M. domestica* and several other selections were highly susceptible to root rot, including *M. × platycarpa* and selections L4, L18, and L19 of *M. coronaria* (means 91–100%, Fig. 1C). Moderate root rot developed in *M. mandshurica*, selection L14 of *M. coronaria*, and *M. baccata* var. *himalaca* (means 46–67%, Fig. 1C). Only *M. halliana* Koehne, *M. × magdebursensis* Hartwig, and *M. sargenti* were highly resistant to both root and crown rot caused by *P. cambivora* (Fig. 1A-C).

In the third experiment (Fig. 2) the relative resistance of four *Malus* spp. evaluated was similar to their relative resistance in the first two experiments. In noninoculated controls, negligible root rot and no crown rot occurred (mean root rot 1–5%, data not shown). Among the inoculated treatments, there was a significant interaction of *Phytophthora* sp. × *Malus* sp. for percent root rot (*P* = 0.001) but not for percentage of crown girdled (*P* = 0.39, after exclusion of treatments with zero-value means and all data from soil infested with *P. cactorum*). Percentage of crown girdled was significantly affected by *Malus* sp. (*P* = 0.0002) but not by *Phytophthora* sp. (*P* = 0.25).

In the third experiment, *M. domestica* (obtained from Schumacher Co.) was the most susceptible of the four selections of *Malus* spp. evaluated, and it developed moderate to severe root and crown rot in soil infested with *P. cactorum*, *P. cambivora*, or *P. cambivora* (Fig. 2). As in the first two experiments, *M. halliana* and *Malus* sp. developed very little root and crown rot in soil infested with *P. cactorum* or *P. cryptogaea* (Fig. 2A and B). However, *M. halliana* sustained greater percentages of crown girdling and root rot caused by *P. cambivora* in the third experiment (means 17 and 19%, respectively, Fig. 2A and B) than in the first two experiments (means 3 and 8%, respectively, Fig. 1A and C). The increased mean severity of disease with *M. halliana* in the third experiment resulted primarily from severe root and crown rot in one of six replicate seedlings. As in the first two experiments, *M. sargenti* was resistant to root and crown rot with each of the three *Phytophthora* sp. used to infest soil in the third experiment (Fig. 2A and B). The third-experiment (and only) evaluation of resistance in *Malus* sp. to *P. cambivora*, *Malus* sp. sustained relatively little root or crown rot (Fig. 2A and B).

**DISCUSSION**

The results of this study indicate that relative resistance to *Phytophthora* root and crown rots in *Malus* spp. is often not the same for different *Phytophthora* spp. Among 12 selections of *Malus* that were evaluated for resistance to all three (*P. cactorum*, *P. cryptogaea*, and *P. cambivora*), six were highly resistant to root and crown rot caused by *P. cactorum*, but only *M. halliana*, *M. × magdebursensis*, and *M. sargenti* were resistant to root and crown rot caused by all three species of *Phytophthora*. Similar variations in relative resistance to the same three *Phytophthora* spp. were revealed in a study that involved clonal apple rootstocks (6).

Such interactions between species of *Phytophthora* and selection of *Malus* suggest that, when seeking resistance to *Phytophthora* root and crown rots, apple rootstock breeders should consider resistance not only to *P. cactorum* but also to other *Phytophthora* spp. that rootstocks may encounter. For example, *M. halliana*, *M. × magdebursensis*, *M. sargenti*, and *Malus* sp. were moderately to highly resistant to *P. cryptogaea* and *P. cambivora*, as well as to *P. cactorum*; these species of *Malus* may offer breeding programs a more encompassing resistance to *Phytophthora* root and crown rots than that offered by other *Malus* spp. with resistance only to *P. cactorum*.

Our results suggest that when resistance of *Malus* spp. to *Phytophthora* is evaluated, researchers should examine...
resistance to root rot as well as to crown rot. Relative resistance to crown rot did not always correspond with relative resistance to root rot. The two measures of crown rot severity, percentage of crown girdled and length of crown rot, provided similar indications of resistance in selections of *Malus* evaluated.

The high level of resistance expressed by *M. × magdебurgensis* in the present study with *P. cactorum* agrees with previous experience (10). Likewise, in a previous work (15), *M. fusca*, *M. loesnitz*, *M. prunifolia* (Willd.) Borkh., *M. baccata* var. *himalaica*, and *M. sargentii* were relatively resistant and *M. domestica* was relatively susceptible to *P. cactorum*.

However, in respect to mortality of seedlings due to *P. cactorum*, our results differ from those obtained by other workers. In the present study, root and crown rot caused by *P. cactorum* in susceptible selections was accompanied by poor growth, leaf discoloration, and at times wilting, but there was virtually no plant mortality. Previous workers reported relatively high rates of mortality among *Malus* spp. that were crown inoculated with zoospores of *P. cactorum*, and mortality rates were used as a measure of resistance to the fungus (9, 15, 22). *M. fusca*, which was comparatively resistant to *P. cactorum* in the present study, suffered a 100% rate of mortality due to the fungus (9).

One possible explanation for differences between our results and those in previous studies is that expressions of resistance varied as a function of age and maturity of seedlings. In earlier studies, seedlings were inoculated at a relatively young stage (two- to three-leaf stage or 10 days to 2-3 wk old) when the root and hypocotyl tissue were morphologically immature. The seedlings of the present study were 7-9 wk old when first exposed to the fungus; at this age the external tissue of the stem bases was brownish in color and apparently lignified. In forest nurseries, the susceptibility of conifer seedlings to damping off caused by *Fusarium oxysporum*, *Pythium* spp., and *Rhizoctonia solani* decreased after a short period of susceptibility following seedling emergence (20). Evaluations of resistance based on plants ≥ 7-9 wk old may not be more accurate than evaluations based on very young seedlings; however, plants ≥ 7-9 wk old are more likely to approximate the mature root and crown tissues of trees that ultimately concern rootstock breeders.

Several additional factors may have contributed to differences in results between this study and previous studies on resistance in *Malus* spp. to *P. cactorum*. Methods of inoculation and environmental conditions that differed between studies may have been important. Genetic variation among collections of *Malus* spp. may explain some of the differences in results. In the case of *M. fusca*, at least, the original provenance of the seed could be a critical factor. Seedlings of *M. fusca* vary from highly susceptible to highly resistant to *P. cactorum*, depending on provenance; the central Oregon type is very susceptible whereas the Alaskan type is resistant (J. N. Cummins, unpublished). Different results could also conceivably follow from specific interactions among isolates of *P. cactorum* and selections of *Malus* that influence disease severity (2). However, this explanation seems unlikely because several isolates of *P. cactorum* were employed among inoculations of the studies (9, 15, 22).

In this study, although known quantities of V8-vermiculite-oat-based inocula were added to soil, no attempt was made to quantify initial soil inoculum densities by bailing or dilution plate methods. With the species of *Phytophthora* that were used, the initial form and density of inoculum propagules in soil should not be expected to be directly meaningful. *Phytophthora cactorum* produces zoospores and sporangia in V8-vermiculite-oat medium, whereas *P. cambivora* and *P. cryptogea* primarily produce only mycelium in the medium (G. T. Browne and S. M. Mirectich, unpublished). However, all three of these *Phytophthora* spp. rapidly produce flushed of zoospores from the inoculum medium during episodes of flooding in soil, and evidence suggests that zoospores are the most important agents of infection (5).

Although there are no reported field studies on comparative resistance to *P. cactorum*, *P. cryptogea*, and *P. cambivora* in the *Malus* selections we evaluated under greenhouse conditions, greenhouse evaluations of resistance to *Phytophthora* spp. in different species of cherry and walnut rootstocks appear to correlate with comparative resistance of the same species of seedling rootstocks in commercial orchards (18,19,23). Field validation of greenhouse assessments of relative resistance in *Malus* spp. to *Phytophthora* spp. would present additional and valuable information.

**LITERATURE CITED**