Testing of Cherry Rootstocks for Resistance to Infection by Species of *Armillaria*

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ABSTRACT

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Ungrafted mahaleb, mazzard, and 17 hybrid *Prunus* rootstocks were inoculated with two intersterile groups of *Armillaria* and the incidence of infection evaluated after 27 mo. None of the interspecific *Prunus* hybrids was immune or highly resistant to infection. Mazzard, M × M 60, and GI 195-1 had the lowest—and mahaleb, Colt, M × M 2, GM 9, and GI 196-4 the highest—incidences of infection. Isolates of the four intersterile groups of *Armillaria* recovered from cherry in Michigan were used to inoculate 1-yr-old Montmorency sour cherry trees grafted on mahaleb and mazzard rootstock. On the basis of the number of grafted trees killed, mazzard was more resistant than mahaleb to infection. The higher resistance of mazzard to *Armillaria* in both experiments was consistent with field observations and supports the use of mazzard rather than mahaleb rootstocks in programs for the control of Armillaria root rot of cherry.

Armillaria root rot is a widespread problem on Montmorency sour cherry trees (*Prunus cerasus* L.) in Michigan. Among isolates of *Armillaria* recovered from orchard trees, four intersterile groups were identified. On the basis of sexual compatibility studies, *A. ostoyae* (Romagn.) Herink. was the predominant species encountered, followed by *A. mellea* sensu stricto (Vahl ex Fr.) Kummer and North American group III of *Armillaria* (5). In 1986, *A. bulbosa* (Barla) Kile & Watling was recovered from a single tree in one orchard (Proffer, *unpublished*).

Most sour cherry trees grown in Michigan are grafted on *P. mahaleb* L. seedlings (mahaleb) (4). A limited number of sour cherry trees, but most sweet cherry trees, are grafted on *P. avium* L. seedlings (mazzard). Field observations indicate that Armillaria root rot is more common on trees grafted to mahaleb than to mazzard rootstock (5,7). No data, however, have been published on the susceptibility of these common cherry rootstocks to *Armillaria*. In addition to mahaleb and mazzard, several interspecific *Prunus* hybrids are now being evaluated at various research stations as possible rootstocks for cherries (1,2,4). Because some of these rootstocks are being planted commercially, information on their susceptibility to *Armillaria* could be useful to rootstock breeders and to cherry growers in production areas where Armillaria root rot is a problem.

The objective of this study was to determine the susceptibility of mahaleb, mazzard, and some representative hybrid cherry rootstocks to infection by *Armillaria*.

MATERIALS AND METHODS

Trial with ungrafted rootstocks. The rootstock clones tested included four *P.*

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mahaleb × *P. avium* hybrids (M × M 2, M × M 39, M × M 46, and M × M 60); three clones from Belgium, GM 9 (*P. incisa* Thunb. × *P. serrula* Franch.), GM 61/1 (*P. dawyckensis* Sealy), and GM 79 (*P. canescens* Bois.); nine clones from Giessen, Germany, GI 148-1 and GI 148-8 (*P. cerasus* × *P. canescens*), GI 154-7 (*P. cerasus* × *P. fruticosa* Pall.), GI 172-7 and GI 172-9 (*P. fruticosa* × *P. avium*), GI 173-9 (*P. fruticosa* × *P. cerasus*), GI 195-1 and GI 195-2 (*P. canescens* × *P. cerasus*), and GI 196-4 (*P. canescens* × *P. avium*); and Colt (*P. pseudocerasus* Lindl. × *P. avium*). All of the clonal hybrids were 1-yr-old liners derived from rooted cuttings or stool-bed layers. All of the above were compared with mahaleb and mazzard 1-yr-old seedling liners.

The rootstocks were planted in May 1985 in 1 × 1 × 0.5 m wooden boxes as described by van Canh (8). Each box, which was open to the ground, was located outdoors between two greenhouses. The sides of each box were lined with plastic. Four 20-cm-diameter tubes, made from sheet metal and open on the ends, were placed vertically in the center of each quadrant before the boxes were filled with sand. One each of the 19 rootstocks was planted at random around two tubes (nine and 10 rootstocks circled each tube). Each rootstock was replicated 10 times across five boxes. Before planting and again each spring, 3 kg of 12-12-12 granular fertilizer was mixed in the top 30 cm of sand. Starting 1 mo later, the plants were fertilized every 2 wk with a solution containing 2 g per box of 20-20-20 fertilizer (R. B. Peters Co., Inc., Allentown, PA). The rootstocks were mulched with about 10 cm of sawdust from rough lumber to reduce the need for frequent watering in summer. The mulch was increased to about 30 cm in late autumn of each year to protect the rootstocks during the winter.

To inoculate the rootstocks, roots of naturally infected sour cherry trees from two orchards, one infected with *A. mellea* sensu stricto and the other infected with North American group III of *Armillaria*, were mixed and placed in the tubes on 18 June 1985 to a depth of about 40 cm and covered with sand. The tubes were then removed to allow the roots of each rootstock to grow in contact with the inoculum. No trees died in 1985, a few died in 1986, and several died in 1987. Trees were examined within 7 days after they died to verify they were infected with *Armillaria*. The surviving trees were removed in August 1987, and the roots and crown were examined for evidence of infection by *Armillaria*. The presence of a fan-shaped, white fungal mat between the bark and the wood was considered evidence that the trees were infected.

Trial with grafted rootstocks. One-year-old Montmorency sour cherry trees on mahaleb and mazzard rootstock were obtained from a commercial nursery (The Nursery Corporation, Hartford, MI). The trunk diameter of the trees was about 1 cm.

Inoculum was prepared using a modification of the procedures of Patton and Riker (3). Branches about 1.2 cm in diameter were collected from mature mahaleb trees in midwinter and cut into 12- to 13-cm segments. Larger branches were reduced to shavings with a planer and to sawdust by grinding the shavings in a Wiley mill. The branch segments were autoclaved for 75 min. To produce inoculum of *Armillaria*, 25 autoclaved branch segments were mixed with a culture medium consisting of 50 g each of oatmeal, cornmeal, mahaleb shavings, and mahaleb sawdust and 300 ml of a 1.5% malt extract solution. The culture medium and branch segments were placed in 36 × 48 cm autoclavable biohazzard bags (American Scientific Products, McGaw Park, IL). Each bag was loosely closed, placed inside a second biohazzard bag, and autoclaved.

After sterilization, each bag was inoculated with a single diploid isolate of *Armillaria* recovered from cherry in Michigan. Four randomly selected isolates of *A. mellea* and *A. ostoyae*, three isolates of group III, and one isolate of *A. bulbosa* were used. Isolates had been grown for 2 wk at 25 C in 60 × 15 mm petri plates containing the enriched malt extract medium of Shaw and Roth (6). Each sterilized bag was inoculated with the contents of three plates and kept at 23–25 C for 14 wk. The trees were inoculated by attaching three colonized branch segments to each rootstock. The segments were placed around the crown of the rootstock, and 2-cm-wide paper tape was wrapped twice around the three segments at their center. Of the 60 replicate trees of each rootstock, 18, 18, 18, and 6 were inoculated with *A. ostoyae, A. mellea*, group III, and *A. bulbosa*, respectively. All trees were inoculated with a single species, but each tree was inoculated with three different isolates, except for those inoculated with *A. bulbosa*, in which a single isolate was used.

In April 1987, the inoculated trees were planted in boxes similar to those used for the ungrafted rootstocks. A divider was inserted across the center of each box, however, to give two 1 × 0.5 × 0.5 m sections per box. Twelve trees were planted in each section, with only a single species of *Armillaria* present per box section. Before planting, 3 kg of 12-12-12 granular fertilizer was mixed in the top 30 cm of sand, and 1 mo after planting, a solution of 20-20-20 fertilizer (2 g per box) was applied every 2 wk. At 3 mo after inoculation, the number of dead trees was recorded. At 4 mo, all trees were harvested and the rootstocks of both dead and living trees were examined for fungal mycelium between the bark and wood, as described for the rootstock trial. The chi-square test of independence was used to evaluate whether there was a significant difference due to rootstock in the number of trees killed by *Armillaria*.

RESULTS

Trial with ungrafted rootstocks. After 27 mo, one to eight trees of each rootstock selection were dead and a few live trees were infected with *Armillaria* (Fig. 1). Rhizomorphs were present on the roots of dead trees and mycelial fans were found beneath the bark. Rhizomorphs were also found on the roots of live trees. Where infection occurred, gummosis was common at the point of penetration and fungal growth was found under the bark. No rootstock selection was free from disease in all 10 replications. Among the two rootstocks in commercial use, mazzard had a lower incidence of *Armillaria* than mahaleb. Although one seedling each of mazzard and mahaleb died, the roots of five mahaleb seedlings were infected with *Armillaria*, whereas those of the remaining mazzard seedlings were healthy. Among the hybrids, M × M 60 and GI 195-1 had low incidences of infection and Colt, M × M 2, GM 9, and GI 196-4 had high incidences. These latter clones were similar to mahaleb in their susceptibility to *Armillaria*.

Trial with grafted rootstocks. After 3 mo, 21.7% of the Montmorency sour cherry trees on mahaleb rootstock and 10.0% of the trees on mazzard rootstock were dead. After 4 mo, 58.4 and 28.3% of the trees were dead on mahaleb and mazzard rootstocks, respectively. The difference between mazzard and mahaleb in the number of trees killed was highly

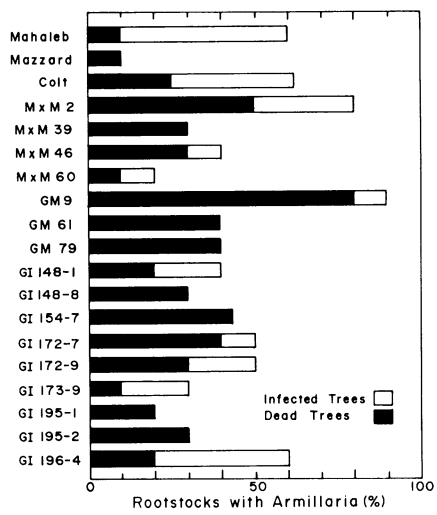

Fig. 1. Incidence of Armillaria root rot on ungrafted cherry rootstocks (10 trees of each) 27 mo after being planted adjacent to infected cherry root and crown tissue. Dead trees were checked for white mycelial fans under the bark within 1 wk after collapsing, and surviving trees were checked at the end of the experiment.

significant ($P = 0.0017$). Rhizomorphs and mycelial fans, characteristic of *Armillaria* infection, were found on all dead trees. On the basis of observations of infected trees, rhizomorphs appeared to provide the primary mode of root infection, rather than direct mycelial penetration at contact points with the inoculum segments. Most of the surviving trees showed evidence of infection. In total, 98.3% of the Montmorency sour cherry trees on mahaleb rootstock and 83.3% of those on mazzard rootstock were dead or infected after 4 mo. Under these experimental conditions, all four *Armillaria* species were highly pathogenic and did not differ significantly in virulence.

DISCUSSION

This appears to be the first study to establish by inoculation that mazzard is less susceptible than mahaleb to *Armillaria*. In both trials, the incidence of *Armillaria* infection was lower on mazzard than on mahaleb rootstocks. The higher resistance of mazzard to Armillaria root rot observed in these inoculation experiments is consistent with the observation in California that mazzard rootstock was distinctly more resistant than mahaleb rootstock (7), and also with the observation in Michigan that the incidence of Armillaria root rot in orchards of sweet cherry on mazzard rootstock was low compared with that in orchards of sour cherry on mahaleb rootstock (5). This study suggests that selecting mazzard over mahaleb rootstock may reduce losses from Armillaria root rot. However, the use of mazzard should be combined with practices to reduce inoculum because high inoculum levels, as used in our second trial, can overcome the inherent resistance of the mazzard rootstock.

This study also provides preliminary information about the susceptibility of experimental cherry rootstocks to Armillaria root rot. None of the rootstocks examined showed greater resistance to *Armillaria* than mazzard. According to our study, there does not appear to be a direct relationship between *Prunus* species parentage and resistance. Instead, resistance appears to be specific to particular clones.

Placing branch segments colonized by *Armillaria* directly on the rootstock before planting provided a reliable method of inoculating trees. Isolates of *Armillaria* not only survived on the inoculum twigs, they also produced rhizomorphs that infected healthy root tissues. Infection and tree mortality data were obtained in one growing season, compared with three growing seasons with the technique used to inoculate ungrafted rootstocks. Thus, it appears that this method would be useful in testing the pathogenicity and virulence of different species of *Armillaria* and in selecting rootstocks for resistance to *Armillaria*. The relative susceptibility of mazzard and mahaleb rootstocks inoculated with *Armillaria*-colonized branch segments is in agreement with field observations. Infection rates, however, were very high under these experimental conditions. The use of shorter or fewer inoculum segments per tree may be required to provide a more distinct separation of rootstocks with differing levels of resistance.

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