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# Resistance of Selected Malus Germplasm to *Rosellinia necatrix*

SANG-BUM LEE,[1] KISUNG KO,[2] AND HERB S. ALDWINCKLE[3]*

**Abstract**

Isolates of *Rosellinia necatrix* from Korea, Japan, and the United States were used to inoculate 1-year-old seedlings from open pollinated 'McIntosh' and 4-year-old M.9 apple rootstock plants to test the isolates' pathogenicity. The Korean and U.S. isolates were more virulent to both plant materials than the Japanese isolate. Three different methods, inserting infected sticks in pots, mixing infested soil into pots, and transplanting seedlings into infested soil, were evaluated for assaying the resistance to *R. necatrix* of *Malus sieversii* seedlings grown from seed collected in the wild in Kazakhstan. The second method gave more equal inoculum pressure and was selected for further tests. The *M. sieversii* seed lot, GMAL 3675 [Plant Introduction (PI) 6005464], showed resistance to the Korean isolate. Among 159 clones of *Malus* germplasm in the apple core collection, 32 clones gave seedlings which had slow development of *R. necatrix* infection or had no necrotic symptoms. In a second test of this germplasm with two fold higher inoculum level, among the selected 32 clones, few seedlings of *M. X domestica* 'E11-24' (PI 589571), *M. X domestica* 'E31-10' (PI 590072), *M. X domestica* 'Redspur Delicious' (PI 589255), *M. X domestica* 'Smith Jonathan' (PI 589845), *M. florentina* "Skopje P2' (PI 589385), *M. micromalus* (PI 594096), *M. prunifolia* 'Naga' (PI 589930), and *M. soulardii* (PI 589391) showed necrotic symptoms of infection, indicating that the parent clones may have some resistance to *R. necatrix*.

## Introduction

While root rot (WRR), a fungal disease of apple caused by *Rosellinia necatrix* (Hartig) Prillieux (4,8), is widely distributed in temperate regions. It has been reported in Asia (China, India, Israel, Japan, Korea, Turkey), the Americas (Argentina, Brazil, Mexico, U.S.), Europe (Austria, Greece, Hungary, Italy), Australia, and Africa (Morocco, South Africa) (3).

*R. necatrix* has a very wide host range that includes 50 plant families (5). Most fruit species, including apple and pear, are susceptible to this disease. WRR has not been controlled successfully on apple although various methods including eradication of infected plants, soil solarization, and methyl bromide treatment have been tried. Biological control has also been studied (1, 7, 10, 11, 12, 13), but without long term success in commercial practice. Sztejnberg and Jabareen (9) reported that 25% of 235 persimmon *(Diospyros kaki)* rootstocks showed resistance in artificial inoculation tests. All apple rootstocks that have been tested were susceptible (4). Lee (6) found no resistance in M.26 and MM.106 apple rootstocks. The objectives of the research reported here were 1) to develop methods to evaluate resistance to WRR in apple germplasm, and 2) to screen a wide selection of *Malus* spp. for resistance to WRR.

## Materials and Methods

### Inoculum.

Three *R. necatrix* isolates were used: KRN, from roots of naturally infected apple from Andong, Kyoung-Buk province, Korea (6); ARN, isolate ATCC 44451 from the American Culture Collection (University Boulevard, Manassas, VA); and JRN, from Biotechnology and Agricultural Chemicals Research Lab, Sumitomo Chemical Co., Takarazuka, Japan. The fungi were maintained on lima bean agar (LMB, Difco, Franklin Lakes, NJ) and then cultured on potato dextrose agar (PDA, Difco) for 14 days for inocu-

[1]Plant Pathology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea; [2]Dept. of Horticultural Sciences, [3]Dept. of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456, USA.

lation experiments. For inoculum, apple sticks (5-10 mm in diameter, 80-100 mm in length) collected from 'McIntosh' apple trees in January were placed on PDA medium, and macerated *R. necatrix* mycelium were kept at 25°C for 30 days. Thirty well-infected sticks bearing the fungal inoculum were mixed with the potting soil (All-Purpose Potting Soil, AGWAY, Syracuse, NY), peat moss, and vermiculite, 20:1:1 (v/v) and incubated in moist conditions under a plastic cover at 25°C for 30 days to create "infested soil."

**Evaluation of virulence of three *R. necatrix* isolates.**

**a) Use of 1-year-old 'McIntosh' seedlings.**

1-year-old seedlings (5-7 mm in diameter) grown from open pollinated (OP) 'McIntosh' were transplanted into 1.5-L plastic pots containing 1 L potting soil. Ten days after transplanting, 100 ml of infested soil of each isolate was placed evenly on the surface of the soil in pots at 1:10 (v/v). The experimental unit was a pot with one seedling. Five pots were tested for each isolate and non-infested soil as a control. Disease development was observed under greenhouse conditions (temperature: 25 ± 2°C, photoperiod: 16 h, relative humidity: 70-80%, two waterings per week). The length of new shoot growth, and disease symptoms were recorded 60 days after inoculation. Plants were removed from pots, soil was washed from the roots, and the roots were observed. Symptoms observed were necrosis and death of leaves and stem, and colonization of roots by cottony, white mycelium.

**b) Use of 4-year-old M.9 apple rootstock plants.**

Dormant M.9 apple rootstock plants stored at 4°C were each transplanted into 4-L pots (18 X 18 cm) containing 3 L potting soil. Ten days after transplanting, 5 infected apple sticks carrying inoculum were inserted into the soil in the pot, spaced 10 cm from the stem. The experimental unit was one plant in a pot. Five plants were tested for each isolate. Disease development on the plants was observed for 120 days. The length of new shoot growth and the disease symptoms as described above were recorded 120 days after inoculation.

**Evaluation of methods for determining resistance to *R. necatrix* using *M. sieversii* populations.**

Seedlings grown from seed collected from 12 wild *M. sieversii* trees in Kazakhstan in 1993, 1995, and 1996 were inoculated with KRN by 3 different methods: a) 50-day-old seedlings were transplanted into the infested soil obtained as described above, b) one infected stick (described above) was inserted into the middle of a line of 10 plants from each of the 12 *M. sieversii* seed lots, c) inoculum soil was mixed into soil in wooden flats in which 50-day-old seedlings were growing. Nine to ten seedlings were evaluated in each test. The percentage of seedlings killed after each treatment was recorded at a) 50, b) 90, and c) 45 days, respectively, after inoculation.

**Evaluation of disease resistance of *Malus* spp. to *R. necatrix*.**

An assessment of the resistance of the genus *Malus* to *R. necatrix* was made by inoculating open-pollinated seedlings from the *Malus* core collection (2). The core collection includes selected domestic apple cultivars, and from one to several clones of most *Malus* species. From 20 to 100 OP apple fruits were harvested from each of 177 clones of the core collection in October, 1996. From 80 to 100 healthy seeds were obtained from each clone. The seeds were washed three times with tap water and kept in distilled water for 24 h. Each population of seed was placed on moistened sterile paper (Fisher Scientific, Pittsburgh, PA) in a 9-cm petri dish. The petri dishes were stored at 0°C for 90 days to break seed dormancy. The seeds from each clone were planted in peat moss: vermiculite, 1:1, in wooden flats (51 X 36 X 7.5 cm). Seedlings were

**Table 1. Pathogenicity or *Rosellinia necatrix* isolates from Korea, United States, and Japan to 1-yr-old 'McIntosh' apple seedlings and 4-yr-old M.9 rootstock plants.**

| | 1-yr-old McIntosh seedlings[z] | | 4-yr-old M.9 plants[y] | |
|---|---|---|---|---|
| *R. necatrix* isolate | # dead plants /# total plants tested[x] | Shoot length (cm)[w] | # dead plants /# total plants tested[v] | Shoot length (cm)[w] |
| KRN (Korea) | 5/5 | –[u] | 5/5 | – |
| ARN (U.S.) | 5/5 | – | 5/5 | – |
| JRN (Japan) | 0/5 | 30 a[t] | 0/5 | 42 a |
| Control | 0/5 | 41 a | 0/5 | 51 b |

[z]Ten days after planting OP McIntosh seedlings in 1 L so9l in 1.5-L pots, 100 ml of the inoculum soil was mixed with the soil in pots at 1:10 (v/v).
[y]Ten days after transplanting of dormant M.9 apple rootstock plants into 3 L soil in 4-L pots, 5 infected apple sticks carrying inoculum were inserted into soil in the pot, spaced 10 cm from the stem.
[x]Shoots wilted ~10 days after inoculation and died ~4 days later.
[v]Shoots wilted ~30 days after inoculation and died ~10 days later.
[w]Mean length of shoots which had grown after inoculation.
[u]It was not possible to measure shoot length since all plants inoculated with KRN and ARN died.
[t]Different letters indicate that shoot length of plants treated with JRN is significantly different from control at p = 0.05 (General Linear Model analysis, SAS Institute, Inc.).

successfully grown from 159 clones. Fifty days after the seeds were planted, *R. necatrix* inoculum (KRN) was added to the soil by evenly spreading 2 L of infested soil on the surface of 10 L of soil in each wooden flat. Observations were conducted every 5 days for 100 days. The reaction level of *Malus* species to *R. necatrix* was calculated and arbitrarily classified into one of three categories: potentially resistant (0 to 30% seedlings with necrotic symptoms), intermediate (31 to 70%), or susceptible (71 to 100%) (Table 4). Another batch of seedlings from those core collection clones that had resistant seedlings in the first test were tested using the same method but with an inoculum level two-fold higher than in the first test.

## Results and Discussion

**Evaluation of virulence of three *R. necatrix* isolates**

**Table 2. Reaction of *M. sieversii* seedlings to inoculation with *R. necatrix* isolate KRN by 3 different inoculation methods.**

| | | Inoculation Methods[z] | | |
|---|---|---|---|---|
| *M. sieversii* seed lot # | Plant introduction # | Transplanting in infested soil[y] | Infected stick[x] | Mixed infested soil[w] |
| GMAL3559 | 600456 | 100 | 56 | 33 |
| GMAL3650 | 600539 | 100 | 56 | 18 |
| GMAL3654 | 600543 | 100 | 60 | 0 |
| GMAL3666 | 600555 | 100 | 40 | 0 |
| GMAL3670 | 600559 | 100 | 22 | 22 |
| GMAL3675 | 600564 | 14 | 40 | 10 |
| GMAL3690 | 600576 | 100 | 50 | 30 |
| GMAL4010 | 600585 | 88 | 50 | 0 |
| GMAL4013 | 600588 | 90 | 70 | 56 |
| GMAL4026 | 600600 | 100 | 22 | 25 |
| GMAL4046 | 600616 | 100 | 0 | 75 |
| GMAL4048 | 600618 | 100 | 22 | 30 |

[z]7-10 seedlings of each seed lot were tested by each inoculation method.
[y]Observed 50 days after inoculation.
[x]Observed 90 days after inoculation.
[w]Observed 45 days after inoculation.

**Table 3. Evaluation of resistance of *Malus* spp. to *R. necatrix*.**

| Core collection clone | PI number | No. of seedlings tested[z] | % of seedlings infected[y] | Category of resistance[x] |
|---|---|---|---|---|
| *M.* X *arnoldiana* | 589222 | 10 | 100 | S |
| *M. asiatica* | 594107 | 9 | 22 | R |
| *M. asiatica* | 589869 | 10 | 90 | S |
| *M. asiatica* | 594099 | 12 | 92 | S |
| *M.* X *atrosanguinea* | 589253 | 7 | 100 | S |
| *M. baccata* 'Alexis' | 589833 | 11 | 100 | S |
| *M. baccata* 'Flexilis' | 437055 | 5 | 100 | S |
| *M. baccata* 'Hansen's #2' | 589838 | 8 | 100 | S |
| *M. baccata* 'Jackii' | 594110 | 11 | 100 | S |
| *M. baccata* 'Mandshurica 2330' | 322713 | 12 | 100 | S |
| *M. baccata* 'Rockii' | 588960 | 9 | 100 | S |
| *M. brevipes* | 589170 | 4 | 100 | S |
| *M. coronaria* | 589976 | 6 | 67 | M |
| *M.* X *domestica* 'Antonovka 1.5 pounds' | 107196 | 8 | 100 | S |
| *M.* X *domestica* 'Antonovka 172670-B' | 589956 | 8 | 100 | S |
| *M.* X *domestica* 'Britegold' | 589726 | 11 | 100 | S |
| *M.* X *domestica* 'Burgundy' | 588835 | 11 | 45 | M |
| *M.* X *domestica* 'Calville Blanc' | 589596 | 7 | 100 | S |
| *M.* X *domestica* 'Chihuahua Gold' | 392311 | 10 | 50 | M[w] |
| *M.* X *domestica* 'Chisel Jersey' | 588806 | 6 | 83 | S |
| *M.* X *domestica* 'Cortland' | 588848 | 10 | 100 | S |
| *M.* X *domestica* 'Cox's Orange Pippin' | 588853 | 10 | 80 | S |
| *M.* X *domestica* 'Dayton' | 590183 | 9 | 89 | S |
| *M.* X *domestica* 'Dorsett Golden' | 589913 | 9 | 78 | S |
| *M.* X *domestica* 'E.8' | 590179 | 9 | 89 | S |
| *M.* X *domestica* 'E7-47' | 590069 | 11 | 82 | S |
| *M.* X *domestica* 'E11-24' | 589571 | 8 | 38 | M[w] |
| *M.* X *domestica* 'E14-32' | 589572 | 8 | 88 | S |
| *M.* X *domestica* 'E29-56' | 590071 | 10 | 80 | S |
| *M.* X *domestica* 'E31-10' | 590072 | 7 | 29 | R |
| *M.* X *domestica* 'E36-7' | 589570 | 8 | 88 | S |
| *M.* X *domestica* 'Ein Shemer' | 280401 | 10 | 100 | S |
| *M.* X *domestica* 'Emilia' | 123989 | 7 | 86 | S |
| *M.* X *domestica* 'Empire' | 588842 | 10 | 90 | S |
| *M.* X *domestica* 'Esopus Spitzenburg' | 588785 | 7 | 100 | S |
| *M.* X *domestica* 'Florina' | 588747 | 12 | 75 | S |
| *M.* X *domestica* 'Fuji Red Sport Type 2' | 588844 | 6 | 83 | S |
| *M.* X *domestica* 'Gala' | 392303 | 9 | 67 | M |
| *M.* X *domestica* 'Golden Delicious' | 590184 | 2 | 100 | S |
| *M.* X *domestica* 'Granny Smith' | 588880 | 12 | 67 | M |
| *M.* X *domestica* 'Gravenstein Washington Red' | 588837 | 4 | 75 | S[w] |
| *M.* X *domestica* 'Haralson' | 589469 | 10 | 60 | M[w] |
| *M.* X *domestica* 'Idared' | 588841 | 12 | 100 | S |
| *M.* X *domestica* 'Ingol' | 589441 | 8 | 100 | S |
| *M.* X *domestica* 'Jonafree' | 589962 | 8 | 100 | S |
| *M.* X *domestica* 'Jonathan' | 590185 | 3 | 67 | M |
| *M.* X *domestica* 'Keepsake' | 589894 | 11 | 100 | S |
| *M.* X *domestica* 'Kimball McIntosh 2-4-4-4' | 589122 | 3 | 100 | S |
| *M.* X *domestica* 'Lady' | 589053 | 10 | 100 | S |

**Table 3. (Continued).**

| Core collection clone | PI number | No. of seedlings tested[z] | % of seedlings infected[y] | Category of resistance[x] |
|---|---|---|---|---|
| *M.* X *domestica* 'Liberty' | 588943 | 9 | 100 | S |
| *M.* X *domestica* 'Macfree' | 589971 | 8 | 100 | S |
| *M.* X *domestica* 'Marshall McIntosh' | 588998 | 23 | 78 | S |
| *M.* X *domestica* 'Medaille d'Or' | 127315 | 9 | 89 | S[w] |
| *M.* X *domestica* 'Mollie's Delicious' | 588981 | 8 | 75 | S |
| *M.* X *domestica* 'Monroe' | 588772 | 8 | 100 | S |
| *M.* X *domestica* 'Murray' | 589486 | 10 | 0 | R |
| *M.* X *domestica* 'Northern Spy' | 588872 | 8 | 100 | S |
| *M.* X *domestica* 'Nova Easygro' | 588838 | 10 | 90 | S |
| *M.* X *domestica* 'Novole' | 590174 | 9 | 100 | S |
| *M.* X *domestica* 'Novosibirski Sweet' | 589478 | 10 | 70 | M |
| *M.* X *domestica* 'Poeltsamaa Winter Apple' | 383515 | 10 | 80 | S[w] |
| *M.* X *domestica* 'PRI 77-1' | 589786 | 9 | 45 | M[w] |
| *M.* X *domestica* 'PRI 333-9' | 589829 | 9 | 77 | S |
| *M.* X *domestica* 'PRI 384-1' | 589780 | 9 | 100 | S |
| *M.* X *domestica* 'PRI 1279-9' | 589791 | 9 | 78 | S |
| *M.* X *domestica* 'PRI 1312-6' | 590079 | 9 | 78 | S |
| *M.* X *domestica* 'PRI 1316-1' | 589776 | 9 | 100 | S[w] |
| *M.* X *domestica* 'PRI 1346-2' | 589785 | 7 | 100 | S |
| *M.* X *domestica* 'PRI 1484-1' | 589790 | 10 | 100 | S |
| *M.* X *domestica* 'PRI 1732-2' | 589946 | 8 | 75 | S |
| *M.* X *domestica* 'PRI 1744-1' | 589789 | 3 | 100 | S |
| *M.* X *domestica* 'PRI 1754-2' | 589794 | 10 | 20 | R |
| *M.* X *domestica* 'PRI 1773-6' | 589807 | 8 | 88 | S |
| *M.* X *domestica* 'PRI 1850-4' | 589792 | 9 | 78 | S |
| *M.* X *domestica* 'PRI 2050-2' | 589819 | 10 | 100 | S |
| *M.* X *domestica* 'PRI 2377-1' | 589812 | 9 | 89 | S[w] |
| *M.* X *domestica* 'PRI 2482-100' | 589795 | 11 | 55 | M[w] |
| *M.* X *domestica* 'Prima' | 589181 | 8 | 88 | S |
| *M.* X *domestica* 'Rambo-Red Summer' | 588798 | 6 | 100 | S |
| *M.* X *domestica* 'Redfree' | 594111 | 8 | 38 | M |
| *M.* X *domestica* 'Redspur Delicious' | 589255 | 9 | 11 | R |
| *M.* X *domestica* 'Reinette Simirenko' | 483257 | 12 | 50 | M[w] |
| *M.* X *domestica* 'Rhode Island Greening' | 589520 | 8 | 100 | S |
| *M.* X *domestica* 'Rome Beauty Law' | 588850 | 9 | 100 | S |
| *M.* X *domestica* 'Rosemary Russet' | 589648 | 8 | 88 | S |
| *M.* X *domestica* 'Russian #12740-7A' | 589835 | 9 | 56 | M |
| *M.* X *domestica* 'Smith Jonathan' | 589845 | 10 | 30 | R |
| *M.* X *domestica* 'Spokane Beauty' | 589006 | 4 | 75 | S |
| *M.* X *domestica* 'Sweet Delicious' | 588955 | 9 | 78 | S[w] |
| *M.* X *domestica* 'Trent' | 589490 | 10 | 80 | S |
| *M.* X *domestica* 'Virginiagold' | 588778 | 10 | 100 | S |
| *M.* X *domestica* 'Wijick McIntosh' | 590186 | 10 | 40 | M |
| *M.* X *domestica* 'Winter Majetin' | 589645 | 8 | 100 | S |
| *M. florentina* | 588868 | 12 | 75 | S |
| *M. florentina* 'Skopje P2' | 589385 | 12 | 0 | R |
| *M. floribunda* | 589741 | 7 | 100 | S |
| *M. floribunda* 821 | 589827 | 9 | 100 | S |
| *M. fusca* | 589975 | 9 | 100 | S |

**Table 3. (Continued).**

| Core collection clone | PI number | No. of seedlings tested[z] | % of seedlings infected[y] | Category of resistance[x] |
|---|---|---|---|---|
| *M. fusca* | 594105 | 11 | 82 | S |
| *M. halliana* | 594112 | 4 | 100 | S[w] |
| *M. hartwigii* | 589420 | 3 | 0 | R |
| *M.* X *hartwigii* | 588757 | 9 | 67 | M[w] |
| *M. honanensis* | 594113 | 7 | 86 | S |
| *M. honanensis* | 589879 | 6 | 67 | S |
| *M. hupehensis* | 594098 | 7 | 86 | S |
| *M. ioensis* | 590015 | 7 | 43 | M |
| *M. ioensis* | 590008 | 7 | 71 | S |
| *M. kansuensis* 'Calva' | 588944 | 9 | 100 | S |
| *M. kirghisorum* | 589380 | 9 | 89 | S |
| *M. mandshurica* | 588753 | 9 | 89 | S |
| *M.* X *magdeburgensis* | 588959 | 7 | 57 | M[w] |
| *M. micromalus* | 594093 | 9 | 100 | S |
| *M. micromalus* | 594092 | 5 | 100 | S |
| *M. micromalus* | 589753 | 8 | 100 | S |
| *M. micromalus* | 594096 | 7 | 71 | S[w] |
| *M. micromalus* | 589955 | 6 | 100 | S |
| *M. orientalis* | 594095 | 12 | 92 | S |
| *M.* X *platycarpa* 'Hoopesii' | 589415 | 9 | 33 | M |
| *M. prattii* | 590045 | 8 | 63 | M |
| *M. prattii* (selfed) | 588933 | 8 | 100 | S |
| *M. prunifolia* | 594102 | 9 | 100 | S |
| *M. prunifolia* '19651' | 589816 | 8 | 100 | S |
| *M. prunifolia* 'Inuringo' | 594103 | 10 | 40 | M |
| *M. prunifolia* 'Microcarpa' | 594109 | 3 | 67 | M[w] |
| *M. prunifolia* 'Naga' (upright) | 589930 | 9 | 0 | R |
| *M. pumila* | 323617 | 10 | 90 | S |
| *M.* X *robusta* 'Korea' | 589003 | 7 | 86 | S |
| *M.* X *robusta* 'Persicifolia' | 589383 | 10 | 100 | S |
| *M.* X *robusta* 'Robusta 5' | 588825 | 9 | 89 | S |
| *M. rockii* | 589421 | 6 | 83 | S |
| *M. sargentii* | 588761 | 9 | 100 | S |
| *M. sieboldii* | 589749 | 3 | 100 | S |
| *M. sieboldii* #387 | 594094 | 12 | 75 | S |
| *M. sieboldii* 'MA#4' | 589958 | 8 | 100 | S |
| *M. sieversii* ssp. *turkmenorum* | 594104 | 8 | 88 | S |
| *M. sikkimensis* | 589390 | 4 | 100 | S |
| *M. soulardii* | 589391 | 6 | 83 | S |
| *M.* X sp. 'Almey' | 588824 | 11 | 91 | S |
| *M.* X sp. 'Demir' | 588883 | 9 | 89 | S |
| *M.* X sp. 'Dolgo' | 588870 | 12 | 92 | S |
| *M.* X sp. 'Jonsib Crab' | 589824 | 10 | 80 | S |
| *M.* X sp. 'Kansas K14' | 588804 | 3 | 67 | M |
| *M.* X sp. 'Kerr' | 588866 | 10 | 0 | R |
| *M.* X sp. 'Prairie Fire' | 589820 | 5 | 80 | S |
| *M.* X sp. 'Roberts Crab' | 437057 | 10 | 100 | S |
| *M.* X sp. 'White Angel' | 588992 | 9 | 11 | R |
| *M. spectabilis* | 594100 | 5 | 100 | S |

**Table 3. (Continued).**

| Core collection clone | PI number | No. of seedlings tested[z] | % of seedlings infected[y] | Category of resistance[x] |
|---|---|---|---|---|
| *M. spectabilis* 'Plena' | 588893 | 7 | 100 | S |
| *M.* X *sublobata* 'Yellow Autumn' | 588922 | 9 | 67 | M |
| *M. sylvestris* | 377590 | 10 | 80 | S |
| *M. sylvestris* | 589382 | 10 | 100 | S |
| *M. sylvestris* | 369855 | 4 | 75 | S |
| *M. toringoides* | 589393 | 9 | 100 | S |
| *M. toringoides* 'Cut-Leaved Crab' | 588920 | 8 | 100 | S |
| *M. transitoria* | 589384 | 11 | 100 | S[w] |
| *M.* X *zumi* 'Calocarpa' | 589840 | 8 | 50 | M |
| *M. yunnanensis* | 589399 | 11 | 100 | S |
| *M. yunnanensis* 'Veitchii' | 589758 | 8 | 75 | S |
| *M. yunnanensis* 'Vilmorin' | 271831 | 6 | 100 | S |

[z]Open pollinated seedlings from apple core collection.
[y]% of seedlings infected was the value calculated from percentage of seedlings with symptoms as a proportion of total seedlings inoculated with *R. necatrix* (KRN). Fifty days after planting seedlings, *R. necatrix* inoculum was incorporated into the soil. The observation was conducted 100 days after inoculation.
[x]R: resistant (0-30 % seedlings with necrotic symptoms), I: Intermediate (30-70 %), S: susceptible (70-100 %).
[w]0 % 70 days after inoculation.

The Korean, U.S., and Japanese *R. necatrix* isolates differed in their pathogenicity both to 1-year-old OP "McIntosh' seedlings (Table 1) and to 4-year-old M.9 rootstock plants (Table 1). Ten days after inoculation the seedlings inoculated with KRN and ARN began to wilt, and 14 days after inoculation the seedlings died (Table 1). The 4-yr-old M.9 plants developed symptoms later than the 1-yr-old seedlings. The 4-yr-old M.9 plants became necrotic and wilted in ~30 days after inoculation, and eventually, died. The surface of the roots of dead plants was covered with strands of cottony, white mycelium as described by Richard (8) and Lee (6). The symptom observed on seedlings and M.9 plants inoculated with JRN was reduced root growth, indicating that the KRN and ARN isolates were more virulent that JRN. However, because these were single isolates, no conclusion can be made about the relative virulence of *R. necatrix* in different regions.

**Evaluation of methods for determining resistance to *R. necatrix* using *M. sieversii* populations.**

The insertion of infected sticks allowed an estimation of the speed of pathogen spread through the soil. However, since the distance of infected sticks to seedlings varied, the inoculum pressure on the seedlings was not uniform. Mixing of infested soil provided more uniform inoculum pressure on the seedlings, as uniform spread of the disease was observed. With the method of replanting seedlings into infested soil, disease resistance was difficult to evaluate since the disease developed suddenly, probably from infection of wounds that occurred during replanting. Therefore, for resistance evaluation, the method of mixing inoculum soil into the soil in the pots 50 days after sowing seeds (4 to 5 true leaves) appeared most effective. This method also is simple to use for the screening and evaluation of many plants.

All *M. sieversii* seed lots had some seedling death in the "infected stick" and "mixed infected soil" methods, whereas all seed lots had greater that 80% seedling death in the "transplanting in infested soil" method (Table 2), except for GMAL 3675 [Plant Introduction (PI) 600564] which had the lowest seedling mortality over the three months. These results indicate that *M. sieversii* GMAL3675 may impart some resistance to *R. necatrix.*

**Evaluation of resistance of *Malus* spp to *R. necatrix.***

**Table 4. Comparison of reactions of seedlings of selected *Malus* spp. clones to low and high levels of inoculum of *R. necatrix* isolate.**

| Core collection clone | PI | N[z] | % seedlings with symptoms Low inoculum[y] | High inoculum[x] |
|---|---|---|---|---|
| M. *asiatica* | 594107 | 10 | 22 | 100 |
| *M.* X *domestica* 'Chihuahua Gold' | 392311 | 6 | 50 | 33 |
| *M.* X *domestica* 'E11-24' | 589571 | 4 | 38 | 25 |
| *M.* X *domestica* 'E29-56' | 590071 | 5 | 80 | 100 |
| *M.* X *domestica* 'E31-10' | 590072 | 4 | 29 | 0 |
| *M.* X *domestica* 'Gravenstein Washington Red' | 588837 | 1 | 75 | 100 |
| *M.* X *domestica* 'Haralson' | 589469 | 8 | 60 | 100 |
| *M.* X *domestica* 'Medaille d'Or' | 127315 | 4 | 89 | 100 |
| *M.* X *domestica* 'Murray' | 589486 | 5 | 0 | 100 |
| *M.* X *domestica* 'Poeltsamaa Winter Apple' | 383515 | 6 | 80 | 100 |
| *M.* X *domestica* 'PRI 1316-1' | 589776 | 5 | 100 | 100 |
| *M.* X *domestica* 'PRI 2377-1' | 589812 | 8 | 89 | 100 |
| *M.* X *domestica* 'PRI 2482-100' | 589795 | 7 | 55 | 100 |
| *M.* X *domestica* 'PRI 77-1' | 589786 | 5 | 44 | 100 |
| *M.* X *domestica* 'Redspur Delicious' | 589255 | 6 | 11 | 17 |
| *M.* X *domestica* 'Reinette Simirenko' | 483257 | 9 | 50 | 56 |
| *M.* X *domestica* 'Smith Jonathan' | 589845 | 10 | 30 | 20 |
| *M.* X *domestica* 'Sweet Delicious' | 588955 | 8 | 78 | 63 |
| *M. florentina* 'Skopje P2' | 589385 | 4 | 0 | 0 |
| *M. halliana* | 594112 | 5 | 100 | 100 |
| *M. hartwigii* | 589420 | 3 | 0 | 33 |
| *M.* X *hartwigii* | 588757 | 7 | 67 | 100 |
| *M. honanensis* | 589879 | 4 | 67 | 100 |
| *M.* X *magdeburgensis* | 588959 | 4 | 57 | 100 |
| *M. micromalus* | 594096 | 3 | 71 | 0 |
| *M. prunifolia* 'Microcarpa' | 594109 | 2 | 67 | 100 |
| *M. prunifolia* 'Naga' (upright) | 589930 | 4 | 0 | 0 |
| *M. seiboldii* #387 | 594094 | 5 | 75 | 100 |
| *M. soulardii* | 589391 | 1 | 83 | 0 |
| *M. transitoria* | 589384 | 3 | 100 | 33 |
| *M.* X sp. 'Kerr' | 588866 | 5 | 0 | 100 |
| *M.* X sp. 'White Angel' | 588992 | 5 | 11 | 40 |

[z]Number of tested seedlings with high inoculum. Number of tested seedlings with low inoculum (see Table 4).
[y]Observed 100 days after inoculation.
[x]Observed 40 days after inoculation.

Disease symptoms were first observed 30 days after inoculation, and symptoms increased rapidly following 60 days after inoculation (Fig. 1). Based on the symptoms observed, each core collection clone was classified into one of three categories, resistant (0 to 30% of seedlings with necrotic symptoms), intermediate (31 to 70%), and susceptible (71 to 100%) (Table 3).

Ten clones with ≤30% seedlings with necrotic symptoms 100 days after inoculation were selected, and a second sample of seeds were germinated and tested in a second evaluation with two-fold higher inoculum concentration. Twenty-two clones with seedlings that only developed symptoms later than 70 days after inoculation, although they were classified as intermediate or susceptible in the first

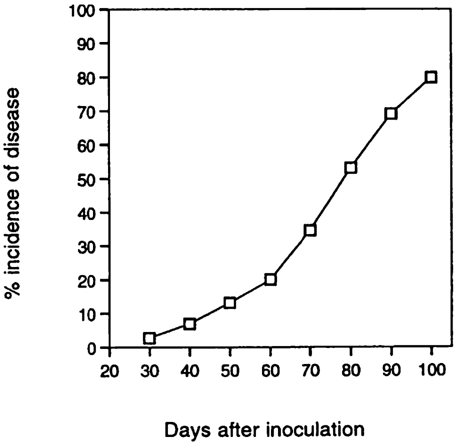


**Figure 1. Disease progress in *Malus* spp. following inoculation with *R. necatrix* isolate KRN. Disease symptoms were observed from 30 to 100 days after inoculation. The number of seedlings with symptoms was divided by the total number of inoculated seedlings to calculate the percentage incidence of disease.**

evaluation (data not shown), were also included in the second evaluation (Table 4).

Most clones whose seedlings showed resistant or intermediate reactions in the first evaluation appeared susceptible, with faster disease development, in the second evaluation (Table 4). However, seedlings of 5 clones [*M. X domestica* 'E31-10' (PI 590072), *M. X domestica* 'Redspur Delicious' (PI 589255), *M. X domestica* 'Smith jonathan' (PI589845), *M. X florentina* 'Skopje P2' (PI 589385), and *M. prunifolia* 'Naga' (PI 589930)] whose seedlings were resistant in the low inoculum test also appeared resistant in the high inoculum test (Table 4). The consistent resistance of seedlings from these five clones in two evaluations indicates that the clones are potential sources of resistance for use in development of *R. necatrix*-resistant apple rootstocks. However, the resistance of the clones' seedlings to natural infection with *R. necatrix* in the field needs to be examined.

Only 3 (*M. X domestica* 'E11-24' (PI 589571), *M. micromalus* (PI 594096), and *M. soulardii* (PI 589391)] of 22 clones whose seedlings showed delayed disease development with low inoculum (data not shown) had resistant reactions. However, there were insufficient seedlings from these clones for any conclusions to be made regarding their resistance.

The parental clones of the 5 seedling populations that showed resistant reactions against *R. necatrix* in both tests may carry factors for resistance, and should be used in crosses and further studies to assess resistance.

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## Roots on Mechanically Pruned Grapes

Mechanical pruning results in increased shoots per vine. Despite significant differences between balanced pruned and mechanically pruned vines in node number, clusters per node, berries per cluster, and yield over 10 years, there were very few differences in root fresh weight, dry weight, soluble carbohydrate or starch levels. From Wample et al. 2000. Amer. J. Enol. Vitic. 51(1):90.

## Peach Leatheriness and Mealiness

After 4 weeks of storage at 0 or 5°C early (20 days) harvested fruit developed more leatheriness but less mealiness than commercial harvested fruits. Late harvest (20 days) fruit did not develop leatheriness but did become more mealy than normal harvested fruit. Storage at 5°C was worse than at 0°C storage. When stored at 10°C for 2 weeks, after which they were senescent, fruit did not develop leatheriness or mealiness regardless of harvest time. Leatheriness caused fruit to be firmer than juicy or mealy fruit. Mealy fruit were as soft as juicy fruit AOC and ACC and polyglacturonase and galactosidase activities were lower and insoluble pectin content was higher in leathery fruit compared to juicy or mealy fruit. From JU et al. 2000. J. Hort. Sci. & Biotech. 25(1):86-91.

## Supercooling of Deciduous Fruit

Water migration from flower primordia of peach and pear to surrounding tissue was observed during freezing. In apple, although the low temperature exotherm of buds without woody stem tissue was not detected, the LT 50 of the bud was cooling rate dependent, and water migration from the primordia to other tissues in the bud was observed during freezing. This indicates that the mechanism of freezing tolerance in apple flower buds are the same as in peach and pear flower buds. Contrarily in persimmon and grape, flower buds without woody stem tissue showed only one exotherm where temperature was closely associated with the LT 50 of the bud. Also both the exotherm temperature of the bud without woody stem tissue and the LT 50 of buds on the woody stem were independent of cooling rate. Persimmon buds showed no water migration during freezing. Provascular strands were found between the bud axis and flower primordia in apple, peach and pear buds but not in persimmon and grape buds. These results indicate that buds of apple, peach, and pear may acquire freezing resistance by extra-organ freezing, but grape and persimmon buds are likely to supercool by themselves. From Kang et al. 1998. J. Hort. Sci. And Biotech 73(2):165-172.