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# Resistance sources to Valsa canker (*Valsa ceratosperma*) in a germplasm collection of diverse *Malus* species

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## Abstract

Although Valsa canker caused by *Valsa ceratosperma* (Tode ex Fr.) Maire is one of the most destructive diseases in apple (*Malus* × *domestica* Borkh.) especially in eastern Asia, information available to help with breeding against Valsa canker in apples is limited. In this work, 53 accessions of diverse *Malus* species and their interspecific hybrids were tested for resistance to *V. ceratosperma*, using an excised shoot assay. Dormant shoots and succulent growing shoots from each accession were inoculated with a virulent isolate AVC-12 of *V. ceratosperma*, and the length of necrosis was measured at 10 days post-inoculation for the dormant shoots and at 7 days post-inoculation for the growing shoots. The lesion length relative to the susceptible control 'Fuji' in dormant shoots ($RL_D$) and to that in growing shoots ($RL_G$) were simple but useful parameters to differentiate between resistant and susceptible accessions. Fourteen accessions from *M. baccata*, *M. florentina*, *M. halliana*, *M. micromalus*, *M. pratii*, *M. sieboldii*, *M. yunnanensis*, *M.* × *floribunda* and *M.* × *platycarpa* gave low $RL_D$ and $RL_G$ values of less than 0.6 and were evaluated as resistant regardless of the difference in the stage of growth. The highest level of resistance was found in *M. sieboldii*. This high level of resistance in *M. sieboldii* was effective against different isolates of *V. ceratosperma*.

**Key words:** *Malus* species — *Valsa ceratosperma* — artificial inoculation

Valsa canker, caused by *Valsa ceratosperma* (Tode ex Fr.) Maire (syn. *Valsa mali* Miyabe et Yamada), is one of the most important diseases of apple; the disease has seriously limited apple production, especially in eastern Asia (Sakuma 1990, Sawamura et al. 1990, Uhm and Sohn 1995). The causal fungus infects the trees through wounds such as the pruning ends or fruit scars. Most new lesions, infected trunks, shoot and fruit scars, appear in spring, and the canker develops rapidly between spring and early summer, and then slowly during the rest of the summer and in the winter. Infection produces localized cankers, and then brings about the death of twigs, limbs or the entire tree afterwards. As the mycelium of the causal fungus is able to invade healthy tissues of bark, phloem and xylem extensively (Tamura and Saito 1982) and because of the perennial nature of the canker, as with stem canker of apple caused by *Botryosphaeria dothidea* and *B. obtusa* (Brown-Rytlewski and McManus 2000), spray applications of fungicides are not always successful for the control of Valsa canker.

Genetic resistance appears to be the most effective and practical method to control the canker. *Valsa ceratosperma* is considered to be equally pathogenic on all cultivated apples (Sawamura et al. 1993); however, high resistance could be present in some *Malus* species and apple rootstocks (Liu et al. 1990, Bessho et al. 1994). The objective of this study was to evaluate resistance levels of diverse *Malus* species, accessions and several apple cultivars to *V. ceratosperma*, using an artificial inoculation test. Additionally some accessions, among which a wide range of resistance exists, were tested against different isolates of *V. ceratosperma* originating from different districts of Japan.

## Materials and Methods

**Plant materials:** Fifty-three accessions of *Malus* species and their interspecific hybrids originating from different geographical locations were inoculated with isolate AVC-12 of *V. ceratosperma*. The reportedly resistant accession *M. sieboldii* 'Sanashi 61' (Bessho et al. 1994) was included as a resistant check and the 'Fuji' apple used as a susceptible check. Ten dormant shoots and 10 succulent growing shoots for each accession were collected from trees in a field of the National Institute of Fruit Tree Science, Morioka, Japan in December and July, respectively. One 12-cm segment was cut from the middle of each shoot and used in the excised shoot assay. Ten twigs for each accession were subjected to the inoculation test.

**Inoculation and disease assessment:** The monoconidial isolate AVC-12 of *V. ceratosperma* (Tode ex Fr.) Maire, originating from an infected apple tree in Aomori Prefecture, Japan, was used. Isolates were multiplied on potato dextrose agar medium for 14 days at 25°C in darkness. The preparation of the inoculum and the method of inoculation followed that developed by Suzaki et al. (1997) with minor modifications. After a 14-day incubation, fungal mycelium ($2.1 \times 10^{-2}$ g) was added into a 100 ml volume of sterilized water and then mildly homogenized for about 30 s. The mixture after homogenization was used as an inoculum (concentration of mycelial solution: $2.1 \times 10^{-4}$ g mycelium per ml) for the excised shoot assay. The distal cut end of each twig segment was subjected to scorching with a flat iron to kill the tissue at the surface of the distal cut end and to ensure easy invasion by the hyphae of the inoculum into the twigs. After scorching, 10 twigs were tied up and placed vertically into a plastic box so that the proximal cut end of each bundle of twigs was about 1 cm deep in the water. A droplet of 20 μl of the inoculum was dripped on to the distal end of each twig at the site of scorching and then the plastic box was immediately covered with a vinyl film to retain the humidity of the inoculated twigs. The twigs were kept in darkness at 25°C for 10 days for the dormant shoot and 7 days for the growing shoot. As non-inoculated controls, five twigs for each accession were prepared by the same procedure described above, and a droplet of 20 μl of sterilized water was put on to the distal end of each twig. These were also kept at 25°C in darkness in humid conditions in the plastic box covered with vinyl film. The lesion length of the necrosis caused by the inoculum was

measured at 10 days post-inoculation for the dormant shoot assay and at 7 days post-inoculation for the growing shoot assay. The non-inoculated controls, which received sterilized water, exhibited no visible symptoms of necrotic lesions in the twigs.

**Stability of resistance against *V. ceratosperma*:** Seven *Malus* accessions, together with resistant and susceptible checks showing a wide range in their degree of resistance, were evaluated with three different isolates. For each accession and isolate, 10–15 twigs were prepared for the excised dormant shoot assay. Three isolates, AVC-12 and AVC-55, originating from an infected apple tree in Aomori Prefecture, Japan, and VC 96-A, originating from Iwate Prefecture, Japan, were used.

## Results and Discussion

In the excised shoot assay, the susceptible control *Malus* × *domestica* 'Fuji' had high values for necrotic lesion lengths (21.3 mm in the dormant shoot assay and 25.1 mm in the growing shoot assay, Table 1), whereas the resistant check *M. sieboldii* 'Sanashi 61' showed the lowest values for lesion lengths (mean length = 4.6 and 4.8 mm in the dormant and growing shoots, respectively) among the *Malus* accessions. The results using dormant shoots agree with those from a previous study (Bessho et al. 1994), in which 'Fuji' was highly susceptible, while a few genotypes of *M. sieboldii*, including 'Sanashi 61', possessed a high level of resistance to Valsa canker among apple rootstocks and *Malus* germplasm collections tested using dormant shoots. Twenty-eight of the fifty-three *Malus* accessions studied, together with the resistant check 'Sanashi 61', displayed lower values for lesions than the susceptible check 'Fuji' (Dunnet's test at $P = 0.05$) in the excised dormant shoot assay. When the growing shoots were used for the inoculation test, 26 accessions showed lower values for mean lesions than 'Fuji.' The results indicated that 23 of the 53 *Malus* accessions had significantly lower values for mean lesions than were observed in 'Fuji' (Dunnet's test at $P = 0.05$) regardless of the difference in the stage of growth (Table 1).

Though resistance studies have not been conducted with inoculations of *V. ceratosperma* using growing shoots, an inoculation test using an excised growing shoot appears to be reliable for the evaluation of the resistance level against *V. ceratosperma*, as well as an inoculation test using excised dormant shoots. These observations are based on the results shown in Table 1. Most of the accessions in the growing shoot assay, however, gave high values for mean lesion lengths in comparison with those of the dormant shoot assay, despite the shorter duration of post-inoculation in the growing shoot assay. This tendency might suggest that the levels of resistance of *Malus* plants to *V. ceratosperma* vary within different kind of plant tissues, dormant shoots and succulent growing ones. The change from actively-growing current shoots to dormant ones in woody plants is characterized mainly by the lignification on the growing shoots; the modification of cell walls by lignin and/or suberin in plant tissue is generally thought to make them more resistant to the compressive forces exerted by pathogens (Bostock and Stermer 1989). In this connection, there are some reports that the lignification of bark tissues is involved with resistance to canker-forming pathogens in woody perennials (Doster and Bostock 1988, Simard et al. 2001). With regard to this study, lignification in the dormant shoots of *Malus* accessions might be associated with the increased levels of resistance to *V. ceratosperma*. Further study will be necessary to determine whether or not the degree of resistance level to *V. ceratosperma* among different kinds of plant tissues is related to lignin and/or suberin formation.

The relative lesion length to the susceptible control'Fuji' was a useful parameter to differentiate between resistant and susceptible accessions regardless of the difference in the stage of growth. The mean length of a lesion of an accession whose relative length to 'Fuji' in dormant shoots ($RL_D$) and that in growing shoots ($RL_G$) had values greater than 0.7 was not significantly different from that of 'Fuji', whereas the lesion value of an accession with an $RL_D$ and $RL_G$ below 0.6 was statistically different from that of 'Fuji' without exception ($P < 0.01$, Table 1). Fourteen accessions, *M. baccata* 'Nikkozumi', *M. florentina*, *M. halliana* 'Hanyaehanakaido' 'Nokaido' 'Tarehanakaido', *M. micromalus* 'HongHaiTang', *M. pratii*, *M. sieboldii* 'Kobanozumi' 'Mo 15' 'Sanashi 61' 'Sanashi 63', *M. yunnanensis* 'Veitchii', *M.* × *floribunda* and *M.* × *platycarpa* gave low $RL_D$ and $RL_G$ values of less than 0.6 and were evaluated as resistant. Ten accessions, *M. asiatica* 'Jiringo' 'Rinki', *M. baccata* 'Mokoto', *M. coronaria* 'Bracteata' 'Charlottae', *M. halliana* 'Hanakaido', *M. ioensis*, *M. kansuensis* 'HeNanHaiTang', *M. sieversii* and *M.* × *arnoldiana* had $RL_D$ and $RL_G$ values of less than 0.7, and at least either the $RL_D$ or $RL_G$ ranged from 0.6 to 0.7; these were classified as moderately resistant. The remaining 29 accessions including major apple cultivars had $RL_D$ and/or $RL_G$ values above 0.7 and were regarded as susceptible.

Significant differences in the degree of resistance to *V. ceratosperma* were observed among both *Malus* species and *Malus* accessions. Interestingly, 7 of the 14 resistant accessions belonged to either *M. halliana* or *M. sieboldii*, which originated in Japan and/or Korea (Way et al. 1990), the most predominant districts for the occurrence of the Valsa canker in the world for apple production. The highest level of resistance was found in *M. sieboldii* among diverse *Malus* accessions. The difference in the level of resistance within accessions belonging to the same *Malus* species appears to reflect the fact that apples are highly heterozygous, which is documented in several isozyme or SSR analyses (Chevreau et al. 1985, Weeden and Lamb 1987, Hokanson et al. 2001, Kitahara et al. 2005, Volk et al. 2005, Guarino et al. 2006). As apples have a gametophytic self-incompatibility system that enforces outbreeding (de Nettancourt 1977), this probably generates high heterozygosity. As a result of this outbreeding, genetic diversity for resistance level to the Valsa canker within *Malus* accessions from the same species has also been generated. All domestic apple cultivars tested, on the contrary, were highly susceptible to the Valsa canker. The scab-resistant 'Prima' and 'Liberty', having *M.* × *floribunda* as an ancestor and carrying $V_f$ (Crosby et al. 1992, Merwin et al. 1994, Durel et al. 2003), were susceptible as well, although *M.* × *floribunda* itself was evaluated as resistant to the Valsa canker. However, some $V_f$ -resistant cultivars, such as 'Releika' and 'Rene', are reported to be resistant not only to apple scab but also to fire blight and bacterial canker (Fischer 2000a,b, Richter and Fischer 2000). Therefore, it may be worthwhile to investigate resistance to the Valsa canker using disease-resistant apple cultivars more extensively and not chosen for the inoculation test in the current study.

There were high positive correlations between lesion lengths caused by isolate AVC-12 and those caused by isolate AVC-55 ($r = 0.995$, $P < 0.01$). Similar findings apply to the lesion lengths caused by isolate AVC-12 and those caused by isolate

Table 1: Length of necrosis on excised shoots of diverse *Malus* accessions inoculated with a virulent isolate, AVC-12 of *Valsa ceratosperma*

| Accession | Species | Origin[1] | Dormant shoot[2] | | Growing shoot[2] | | Evaluation[3] |
|---|---|---|---|---|---|---|---|
| | | | Mean length of lesion (mm) | $RL_D$ | Mean length of lesion (mm) | $RL_G$ | |
| Fuji | *M.* × *domestica* Borkh. | (Susceptible check) | 21.3 | 1.00 | 25.1 | 1.00 | Susceptible |
| M. angustifolia | *M. angustifolia* Michx. | North America | 16.0 | 0.75 | 16.1** | 0.64 | Susceptible |
| Daito 2 | *M. asiatica* Nakai | North China, Korea | 18.0 | 0.85 | 18.0 | 0.72 | Susceptible |
| Jiringo | *M. asiatica* Nakai | North China, Korea | 13.1** | 0.62 | 13.5** | 0.54 | Moderately resistant |
| Rinki | *M. asiatica* Nakai | North China, Korea | 13.9** | 0.65 | 15.7** | 0.63 | Moderately resistant |
| Waringo | *M. asiatica* Nakai | North China, Korea | 16.5 | 0.77 | 17.2* | 0.69 | Susceptible |
| Mandschurica 1 | *M. baccata* Borkh. | North China, Japan, Korea | 15.3 | 0.72 | 18.0 | 0.72 | Susceptible |
| Mokoto | *M. baccata* Borkh. | North China, Japan, Korea | 13.2** | 0.62 | 16.9** | 0.67 | Moderately resistant |
| Nikkozumi | *M. baccata* Borkh. | North China, Japan, Korea | 6.0** | 0.28 | 14.5** | 0.58 | Resistant |
| ShanDingZi 1 | *M. baccata* Borkh. | North China, Japan, Korea | 12.9** | 0.61 | 18.6 | 0.74 | Susceptible |
| ShanDingZi 3 | *M. baccata* Borkh. | North China, Japan, Korea | 15.2 | 0.71 | 17.4 | 0.69 | Susceptible |
| Bracteata | *M. coronaria* Mill. | North America | 13.8** | 0.65 | 15.4** | 0.61 | Moderately resistant |
| Charlottae | *M. coronaria* Mill. | North America | 14.5 | 0.68 | 16.2** | 0.64 | Moderately resistant |
| M. florentina | *M. florentina* (Zucc.) Schneid. | Europe | 7.0** | 0.33 | 11.0** | 0.44 | Resistant |
| ChuiSiHaiTang | *M. halliana* Koehn. | Japan | 15.4 | 0.72 | 25.5 | 1.02 | Susceptible |
| Hanakaido | *M. halliana* Koehn. | Japan | 9.4** | 0.44 | 15.7** | 0.63 | Moderately resistant |
| Hanyaehanakaido | *M. halliana* Koehn. | Japan | 12.0** | 0.56 | 11.2** | 0.45 | Resistant |
| Nokaido | *M. halliana* Koehn. | Japan | 7.8** | 0.37 | 11.7** | 0.46 | Resistant |
| Tarehanakaido | *M. halliana* Koehn. | Japan | 10.5** | 0.49 | 12.2** | 0.49 | Resistant |
| M. honanensis | *M. honanensis* Rehd. | Central China | 18.0 | 0.85 | 19.5 | 0.78 | Susceptible |
| HuBeiHaiTang 83051 | *M. hupehensis* Rehd. | Central China | 15.6 | 0.73 | 20.8 | 0.83 | Susceptible |
| M. ioensis | *M. ioensis* Brit. | North America | 11.8** | 0.55 | 16.8** | 0.67 | Moderately resistant |
| HeNanHaiTang | *M. kansuensis* Schneid. | North-western China | 13.7** | 0.64 | 13.2** | 0.52 | Moderately resistant |
| HongHaiTang | *M. micromalus* Makino | South-eastern China, Korea | 11.4** | 0.54 | 14.1** | 0.56 | Resistant |
| LiJiangHaiTang | *M. micromalus* Makino | South-eastern China, Korea | 20.6 | 0.97 | 24.4 | 0.97 | Susceptible |
| M. pratii | *M. pratii* (Hemsl.) Schneid. | South-western China | 11.0** | 0.52 | 11.0** | 0.44 | Resistant |
| Kobanozumi | *M. sieboldii* Rehd. | Japan, Korea | 11.6** | 0.54 | 12.3** | 0.49 | Resistant |
| Miyamakaido | *M. sieboldii* Rehd. | Japan, Korea | 13.4** | 0.63 | 23.3 | 0.93 | Susceptible |
| MO 15 | *M. sieboldii* Rehd. | Japan, Korea | 5.0** | 0.23 | 5.8** | 0.23 | Resistant |
| Sanashi 61 | *M. sieboldii* Rehd. | Japan, Korea (Resistant check) | 4.6** | 0.22 | 4.8** | 0.19 | Resistant |
| Sanashi 63 | *M. sieboldii* Rehd. | Japan, Korea | 11.5** | 0.54 | 10.2** | 0.41 | Resistant |
| M. sieversii | *M. sieversii* (Lodeb.) Roem. | North-western China | 14.0* | 0.66 | 15.4** | 0.61 | Moderately resistant |
| M. sikkimensis | *M. sikkimensis* Koehn. | Himalaya | 14.5 | 0.68 | 19.0 | 0.76 | Susceptible |
| HaiTangHua | *M. spectabilis* Borkh. | Not identified | 12.3** | 0.58 | 19.0 | 0.76 | Susceptible |
| BianYeHaiTang 83034 | *M. toringoides* Hughes. | Not identified | 11.0** | 0.52 | 19.1 | 0.76 | Susceptible |
| Miyagi 1 | *M. tschonoskii* Schneid. | Japan | 16.3 | 0.76 | 20.3 | 0.81 | Susceptible |
| M. yunnanensis | *M. yunnanensis* Schneid. | South-western China | 17.0 | 0.80 | 19.6 | 0.78 | Susceptible |
| Veitchii | *M. yunnanensis* Schneid. | South-western China | 11.5** | 0.54 | 14.9** | 0.59 | Resistant |
| M. arnoldiana | *M.* × *arnoldiana* Sarg. | | 14.1* | 0.66 | 14.9** | 0.59 | Moderately resistant |
| M. floribunda 88071 | *M.* × *floribunda* Sieb. | | 9.5** | 0.45 | 9.4** | 0.37 | Resistant |
| M. gloriosa | *M.* × *gloriosa* Lemoine | | 15.8 | 0.74 | 19.3 | 0.77 | Susceptible |
| M. hartwigii | *M.* × *hartwigii* Koehn. | | 16.8 | 0.79 | 20.0 | 0.80 | Susceptible |
| M. platycarpa 73031 | *M.* × *platycarpa* Rehd. | | 9.8** | 0.46 | 11.7** | 0.47 | Resistant |
| M. robusta Bailey | *M.* × *robusta* Rehd. | | 14.6 | 0.69 | 20.5 | 0.82 | Susceptible |
| Robusta 5 | *M.* × *robusta* Rehd. | | 16.2 | 0.76 | 24.3 | 0.97 | Susceptible |
| Scheideckeri | *M.* × *scheideckeri* Zabel | | 9.3** | 0.44 | 18.3 | 0.73 | Susceptible |
| M. soulardii | *M.* × *soulardii* Brit. | | 15.4 | 0.72 | 20.0 | 0.80 | Susceptible |

Table 1: Continued

| Accession | Species | Origin[1] | Dormant shoot[2] | | Growing shoot[2] | | Evaluation[3] |
|---|---|---|---|---|---|---|---|
| | | | Mean length of lesion (mm) | $RL_D$ | Mean length of lesion (mm) | $RL_G$ | |
| Gala | *M.* × *domestica* Borkh. | | 17.8 | 0.84 | 25.8 | 1.03 | Susceptible |
| Golden Delicious | *M.* × *domestica* Borkh. | | 17.0 | 0.80 | 20.1 | 0.80 | Susceptible |
| Jonathan | *M.* × *domestica* Borkh. | | 20.9 | 0.98 | 24.6 | 0.98 | Susceptible |
| Liberty | *M.* × *domestica* Borkh. | | 18.5 | 0.87 | 19.9 | 0.79 | Susceptible |
| Prima | *M.* × *domestica* Borkh. | | 18.3 | 0.86 | 23.4 | 0.93 | Susceptible |
| Starking Delicious | *M.* × *domestica* Borkh. | | 23.7 | 1.11 | 25.9 | 1.03 | Susceptible |

$RL_D$: relative lesion length to 'Fuji' in dormant shoots (mean lesion length of the respective accession/21.3), $RL_G$: relative lesion length to 'Fuji' in succulent growing shoots (mean lesion length of the respective accession/25.1).
[1]Origin of the primary *Malus* species described by Way et al. (1990).
[2]Dormant shoots were collected and inoculated in December, and growing shoots in July.
[3]Susceptible: $RL_D > 0.70$ or $RL_G > 0.70$; moderately resistant: $0.60 \leq RL_D$ and/or $RL_G \leq 0.70$; resistant: $RL_D$, $RL_G < 0.60$.
*and **differ significantly from the susceptible check 'Fuji' (Dunnet's test at $P = 0.05$ and $P = 0.01$).

VC 96-A ($r = 0.985$, $P < 0.01$), and the lesion lengths caused by isolate AVC-55 and those caused by isolate VC 96-A ($r = 0.990$, $P < 0.01$) when nine accessions from diverse *Malus* species showing a wide range in their degree of resistance were tested for the excised dormant shoot assay (data not shown). Although little is known about the specific interaction between *Malus* genotypes and isolates of *V. ceratosperma*, the results suggested that there was no evidence for the existence of specific races virulent to *M. sieboldii*.

Clearly, several accessions from *M. sieboldii*, *M. halliana*, *M. florentina* and *M.* × *floribunda* were demonstrated to possess a good level of resistance against *V. ceratosperma*; 'Mo 15' and 'Sanashi 61' from *M. sieboldii* were evaluated and found to have a particularly high level of resistance, which makes them useful as breeding materials for resistance breeding against the Valsa canker. In the process of selecting resistant plants against *V. ceratosperma* in the progenies derived from the resistant *Malus* species mentioned above, the values of the relative lesion length to the susceptible control 'Fuji' are acceptable as a simple but reliable parameter. To date, a number of *Malus* species have been used worldwide to improve certain traits in the cultivated apple. As a result, it was found that interspecific hybridizations between cultivated apples and a wide range of *Malus* species was successful (Korban 1986, Bus et al. 2000, Fischer et al. 2003). Among the resistant *Malus* species against Valsa canker, *M. sieboldii* is known to possess the highest level of resistance to fire blight (Gardner et al. 1980) and powdery mildew (Schuster 2000), *M. florentina* is resistant to powdery mildew (Schuster 2000), and families originating from *M. baccata* are resistant to fire blight (Gardner et al. 1980, Luby et al. 2002); consequently a long-term apple breeding programme for multiple disease resistance can be justified using a resistant accession as a parent.

The strong focus of the resistance breeding programme is currently put on resistance to scab (*Venturia inaequalis*), as is the case in the apple-breeding programme at the National Institute of Fruit Tree Science (NIFTS) in Japan. In this connection, new scab-resistant cultivars with attractive fruit appearance and/or quality (e.g. Fischer 2000a, Janick et al. 2004a,b, Khanizadeh et al. 2003, Korban et al. 2003, Laurens et al. 2005) are worthwhile not only for testing their field performance in the environmental conditions at several apple production districts in Japan but also for consideration as parents in future breeding at NIFTS to develop dessert apple cultivars with multiple resistance.

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