# Union Aberration of Sweet Cherry on *Prunus mahaleb* Rootstock Associated with X-disease

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**ABSTRACT**

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Several sweet cherry (*Prunus avium*) scions, including cultivars Bing and Sam, on mahaleb roots (*P. mahaleb*) developed pits and grooves (aberration) in the wood at the union of the scion and rootstock a year after having been graft-inoculated with tissue from trees with symptoms of X-disease. Graft inoculations of Bing trees on Colt (*P. avium* × *P. pseudocerasus*) rootstock produced chronic X-disease symptoms consisting of small leaves and small fruit with short pedicels, but no union aberration. Several symptomatic indicator trees, but not healthy trees, were confirmed to contain X-disease mycoplasmalike organisms (XMLOs) by hybridization assay using a radioactive-labeled DNA probe specific for XMLO. Assays of X-diseased cherry trees for tomato ringspot virus by indirect ELISA and bud grafts onto *P. tomentosa* were negative.

X-disease, presumed to be caused by a mycoplasmalike organism (MLO) (3), has virtually eliminated sweet cherry orchards in the Green Valley and Napa Valley regions of California and continues to cause problems in San Joaquin County, the major sweet cherry production area in California. X-disease infection in sweet cherry on roots of mazzard (*Prunus avium* L.) and Stockton morello (*P. cerasus* L.) causes small leaves, poor shoot growth, and fruit that are either small and rounded with long pedicels (Napa Valley strain) or small and pointed with short pedicels (Green Valley strain) (1). Onset of fruit and canopy symptoms usually occurs during the second year after onset of infection. The predominant fruit symptom observed in San Joaquin County is caused by the Green Valley strain.

In contrast to the chronic symptoms produced on the above-mentioned rootstocks, diseased sweet cherry trees on mahaleb (*P. mahaleb* L.) roots are difficult to diagnose because affected trees collapse suddenly and fruit symptoms are usually absent. Also, decline and death of sweet cherry/mahaleb trees are sometimes associated with Phytophthora crown and root rot, rodent damage to underground bark tissues, and abiotic factors such as poorly drained soils or improper orchard management. Approximately 85% of the sweet cherry trees cultivated in California are grown on mahaleb seedling rootstock. In the course of examining graft-inoculated indicator trees, we observed a union aberration symptom, consisting of pits and grooves in the xylem. The consistent induction of such symptoms by different sources of X-disease inoculum was investigated to establish a causal relationship.

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## MATERIALS AND METHODS

During 1985, samples of fruit and limbs of sweet cherry showing symptoms of X-disease were obtained from orchards in San Joaquin County. Later, to confirm the visual diagnosis, several orchards in the county were visited and bud sticks were collected for purposes of bioassay. Eight bud sticks were taken from each of 20 mature Bing trees and one Early Burlat tree showing canopy and/or fruit symptoms that were typical of X-disease. Eleven collections were from diseased trees on mahaleb and five each were from trees on roots of mazzard and Stockton morello. A bud chip from each bud stick (eight per indicator tree) was inserted into the scion portion of 8-yr-old Bing or Sam sweet cherry cultivars on mahaleb roots. Five indicator trees (four Bing and one Sam) served as uninoculated controls throughout the test period. Symptoms were read 12–36 mo after inoculation.

In 1986 and 1987, surveys of three young orchards (trees in the two- to five-leaf stage of growth) planted to Bing on Colt (*P. avium* × *P. pseudocerasus*) rootstock revealed several scattered trees that showed a reddish coloration of the upper leaf surface and drooped leaves held in an overlapping fashion. When the same trees were examined the next growing season, the leaves were small, cupped slightly upward, and had a wavy margin. Some fruit were produced on shortened pedicels. To determine if these symptoms were associated with X-disease, bioassays were done. Large limbs or bud sticks from one healthy and six symptomatic trees were sampled and graft-inoculated, using three bark patches or bud chips per collection, onto two or four indicator trees of Bing/mahaleb (two- and three-leaf stage of growth) and/or Bing/Colt (three-leaf stage of growth). These indicator trees were read after a 12- to 24-mo incubation period.

To determine if pits and grooves would develop when the scions of other sweet cherry selections on mahaleb seedling rootstock are inoculated, 10 advanced selections from the University of California, Davis, (UCD) cherry breeding program and cultivars Sweet Ann, Early Burlat, and Bing were high-worked with multiple scion buds onto mahaleb seedlings. The Sweet Ann cultivar is immune to X-disease (13). Three inoculum chips were inserted in each scion shoot (three to seven shoots inoculated per selection or cultivar). Uninoculated scion shoots (controls) consisted of one to five shoots per selection or cultivar. Inoculated shoots were examined 1 yr after inoculation. For those selections and cultivars remaining healthy, the inoculation process was repeated on the same shoots and examined 1 yr later.

To confirm the presence of the X-disease MLO (XMLO) in symptomatic indicator trees, DNA hybridization assays (5) were conducted. DNA extracts prepared from leaf petioles of symptomatic indicator trees of Bing/Colt (infected by two different inocula), a UCD cherry selection/mahaleb showing severe union aberration (selection 11-40, tree 3, Table 1), budling propagations from X-diseased Bing/mazzard and Bing/Colt (shoots showing stunted growth), several healthy Bing/mazzard, and a celery plant with X-disease were spotted onto a nitrocellulose filter (BA 85, Schleicher and Schnell) and probed with $^{32}$P-labeled XMLO-specific DNA (5). The probe, referred to as pWX-1 (5), contains a 4.1-kb insert of the WX-MLO chromosome. When digested to completion with *Eco*RI and *Hin*dIII, it yields five fragments ranging from 0.30 to 0.48 kb and one 2.1-kb fragment (B. C. Kirkpatrick, *personal communication*). The dot-blot filters were subsequently exposed to X-ray film and developed as previously described (5).

Because tomato ringspot virus (TmRSV) incites a union disorder in apple (*Malus sylvestris* Mill.) called apple union necrosis and decline (AUND) (10) and a union disorder in prune (*P. domestica* L.) called prune brownline (PBL) (4), bioassays and seroassays were performed with bud grafts and tissue extracts, respectively, in an attempt to detect this nepovirus in the X-disease cherry trees.

For enzyme-linked immunosorbent assays (ELISA), tissues were extracted in carbonate buffer (1:5, w/v) (15), spotted in duplicated wells in microtiter plates, and processed by the indirect procedure (8). An IgG preparation specific for the PBL isolate of TmRSV (TmRSV-PBL) and a culture of the yellow bud mosaic strain of TmRSV (TmRSV-YBM) were provided by A. Rowhani (UCD). The IgG fraction was used as the probe immunoglobulin. Peroxidase-labeled goat antirabbit IgG and *o*-phenylenediamine substrate (both from Sigma Chemical Co., St. Louis, MO) were added as previously described (15).

Leaf and bark extracts were prepared from the scion of a Bing/Colt tree and the scion and rootstock of UCD cherry selection 11-40/mahaleb (Table 1, tree 3). Both source trees were symptomatic for X-disease and had indexed negative on Shirofugen flowering cherry (*P. serrulata* Lindl.), an indicator for prune dwarf and Prunus necrotic ringspot viruses (*unpublished data*). Also, extracts were prepared from peach (*P. persica* (L.) Batsch) and cucumber (*Cucumis sativus* L.) infected with TmRSV-YBM. Healthy cherry, peach, and cucumber extracts were included as negative controls.

For bioassays, the above collections were bud-inoculated onto potted seedlings of *P. tomentosa* Thunb., an indicator of TmRSV (6). Four bud chips were grafted onto each of four *P. tomentosa* plants per collection. Four *P. tomentosa* plants served as nongrafted controls. The indicators, after 6 wk of incubation, were indexed on *Chenopodium quinoa* Willd. Here, succulent leaf tissues were extracted in nicotine-phosphate buffer and rubbed onto corundum-dusted leaves of the herbaceous indicator plants (14). ELISA tests also were done on extracts of the *C. quinoa* plants.

## RESULTS

After 12–36 mo of incubation, 18 of 21 indicator trees grafted in 1985 initially produced a light green canopy, with the terminal leaves folded upward longitudinally and with a reddish coloration of the underside of the leaves. Later, during the summer months, several symptomatic trees began to collapse and die. Eventually all except three grafted trees and the five ungrafted controls died. Before the affected trees collapsed totally, the unions were examined in the field by means of an arch punch (37 mm diameter) (C. S. Osborne & Co., Harrison, NJ) and hammer to remove bark cores from several places above, below, and including the union area to reveal the underlying woody cylinder. Bark cores from all affected trees showed a diffuse brown line in the phloem tissue along the union. Correspondingly, the unions in the xylem (woody cylinder) showed numerous pits and grooves (union aberration) (Fig. 1, Table 2). Such symptoms were evident only at the scion/rootstock junction and were not observed elsewhere on diseased trees. The unions of the remaining symptomless grafted- and control-indicator trees appeared normal (Fig. 2A).

Inocula from diseased orchard trees on Colt roots, which were graft-inoculated in 1986 and 1987 on Bing/mahaleb indicator trees, also caused tree collapse and union aberration symptoms (Fig. 2B, Table 2). To facilitate the examination of the whole union on young indicator trees, the union portion of the tree was autoclaved for 10–20 min and, prior to cooling, the bark was removed. On Bing/Colt indicator trees graft-inoculated in 1986, two diseased collections induced symptoms (2 yr later) similar to those described for the original orchard trees (Table 2). All controls growing on roots of mahaleb or Colt remained healthy. The union of diseased Bing/Colt trees appeared normal.

After two attempts, three sweet cherry selections and Sweet Ann proved refractory to X-disease by graft inoculation (Table 1). Among the remaining seven selections plus the named scions Bing and Early Burlat, one or more inoculated

**Table 1.** Response of University of California, Davis, selections and cultivars of sweet cherry to X-disease inoculations

| Selections and cultivars | Number of trees[a] | Shoots diseased/inoculated shoots[b] (no.) | Noninoculated shoots diseased/noninoculated shoots[b] (no.) |
|---|---|---|---|
| **Selections** | | | |
| 1-19 S | 1 | 1/4 | 0/2 |
| 1-24 SE | 1 | 0/3 | 0/2 |
| 2-10 NE | 1 | 3/3 | 0/1 |
| 9-73 | 1 | 0/2 | 0/1 |
| | 2 | 0/1 | 0/1 |
| | 3 | 2/2 | 0/2 |
| 9-75 | 1 | 2/2 | 0/1 |
| | 2 | 2/3 | 0/1 |
| 11-40 | 1 | 1/2 | 0/1 |
| | 2 | 1/2 | 0/1 |
| | 3 | 3/3 | 0/1 |
| 12-1 | 1 | 0/2 | 0/2 |
| | 2 | 0/1 | 0/1 |
| 12-28 | 1 | 2/2 | 0/1 |
| | 2 | 0/1 | 0/1 |
| 13-22 | 1 | 0/2 | 0/1 |
| | 2 | 1/2 | 0/1 |
| 16-17 | 1 | 0/2 | 0/1 |
| | 2 | 0/2 | 0/1 |
| **Cultivars** | | | |
| Sweet Ann | 1 | 0/1 | 0/1 |
| | 2 | 0/2 | 0/1 |
| Early Burlat | 1 | 1/2 | 0/1 |
| | 2 | 0/2 | 0/1 |
| Bing | 1 | 2/3 | 0/1 |

[a] Multiple scion buds per selection and cultivar were T-budded onto one to three mahaleb (*Prunus mahaleb*) rootstocks, and two or more budling shoots per rootstock were encouraged to develop.

[b] Selected shoots received three inoculum buds each. One or more control shoots on the same tree were not inoculated. Inoculations were repeated on the same shoots for those selections and cultivars remaining healthy after a 12-mo incubation. Diseased, but not healthy, shoots showed pits and grooves at the scion/rootstock union.

**Fig. 1.** An exposed symptomatic union of a mature Bing sweet cherry/mahaleb tree infected by X-disease; the tree had been graft-inoculated 12 mo earlier.

shoots developed pits and grooves at the scion/mahaleb rootstock junction.

With the DNA hybridization assays, all diseased extracts tested positive, whereas healthy controls were negative.

Seroassays showed positives for leaf extracts of peach infected with TmRSV-YBM (av. $A_{450nm}$ of 0.47) and infected cucumber (av. $A_{450nm}$ of 0.46). $A_{450nm}$ values for extracts of X-disease cherry, healthy cherry, peach, and cucumber were negative 0.04, 0.03, 0.10, and 0.18, respectively. With bioassays on *P. tomentosa*, chlorotic spots and line pattern were produced on plants grafted with TmRSV-YBM inocula after a 3-wk incubation. After 6 wk, symptoms became more intense and readily evident in all *P. tomentosa* plants receiving TmRSV-YBM inocula. No symptoms were apparent on the remaining indicator plants grafted with the X-disease cherry collections and nongrafted controls. Likewise, subsequent assays of all *P. tomentosa* plants on *C. quinoa* resulted in virus transmissions from only the four symptomatic plants grafted with bud chips containing TmRSV-YBM. Symptoms on *C. quinoa* consisted of primary lesions followed by epinasty of the apical leaves and stem. ELISA values for symptomatic and healthy appearing *C. quinoa* extracts were $A_{450nm}$ of 0.49 and 0.09, respectively.

## DISCUSSION

In the early literature, investigations of internal symptoms associated with X-disease sweet cherry/mahaleb trees were largely confined to bark tissues, where phloem degeneration (9) and/or sieve tube necrosis (11) were reported. Anatomical changes that may have occurred in the xylem (particularly at the scion/rootstock junction) were not mentioned. In the present study, as a number of sweet cherry scions were tested and responded in a similar manner, it appears that union aberration is a result of an interaction between the pathogen and the mahaleb rootstock, which appears to influence the subsequent growth of the scion tissue (Fig. 2B). Some variation in the intensity of the pathogen-root response may occur, however. For example, Gilmer et al (2) and Rawlins and Thomas (9) reported that some mahaleb seedlings were systemically infected by graft inoculation and the X-disease pathogen was transmitted from mahaleb to other indicator hosts. Whether commercially planted trees on genetically similar, susceptible mahaleb seedlings would react with chronic infections, i.e., without rapid tree collapse and union aberration, remains to be determined. In general, trees on mahaleb infected with X-disease react in an acute fashion, suggestive of a hypersensitive response of the rootstock to the pathogen. Although this is the first formal report of union aberration, this symptom is currently being used to aid in the field diagnosis of X-disease infections in collapsing sweet cherry trees in California.

In several *Prunus* spp., TmRSV is known to incite pits and grooves in the xylem that often extend beyond (above and/or below) the union area. The disease is known as Prunus stem pitting (6,12). Also, and depending on the host species, TmRSV infections induce symptoms confined to the junction of the scion and rootstock, e.g., PBL and AUND. With X-disease cherry samples, however, negative bioassays and seroassays strongly suggest that the X-disease MLO, and not TmRSV, is the likely incitant of union aberration. Noninvolvement of TmRSV is further supported by the fact that on multishoot-bearing mahaleb trees (Table 1), only some bud-inoculated shoots, but not uninoculated ones on the same tree, developed union aberration. As TmRSV

**Table 2.** Symptoms and efficacy of graft inoculation in transmitting X-disease to Bing cherry trees on mahaleb and Colt rootstocks

| Year inoculated | Source of inoculum (Bing trees on the various rootstocks) | Indicator trees: Number of Sam or Bing/mahaleb trees with union aberration | Indicator trees: Number of Bing/Colt trees with symptoms of X-disease |
|---|---|---|---|
| 1985[a] | On mahaleb | 8/11[b] | ...[c] |
| | On mazzard | 5/5 | ... |
| | On Stockton morello | 5/5 | ... |
| | Healthy controls | 0/5 | ... |
| 1986[d] | On Colt (no. 1) | 1/4 | 4/4[e] |
| | On Colt (no. 2) | 1/4 | 2/4 |
| | Healthy controls | 0/4 | 0/4 |
| 1987[f] | On Colt[g] | 7/8 | ... |
| | Healthy controls | 0/4 | ... |

[a] Eight bud chips per collection were grafted into the scion portion of each indicator tree at the eight-leaf stage of growth. Five uninoculated indicator trees were included. Trees were read after 12–36 mo of incubation.

[b] The number of indicator trees developing union aberration symptoms (numerator) and the number of collections used as inoculum sources (denominator), except control trees, which were not graft-inoculated.

[c] Not tested.

[d] Three bark patches grafted per collection per indicator tree at the three-leaf stage of growth. Control trees were grafted with a healthy collection. Trees were read after 12–24 mo of incubation.

[e] These indicator trees expressed leaf and fruit symptoms of X-disease in 1988 (number diseased trees/number inoculated). Unions were normal.

[f] Three bud chips grafted per collection per indicator tree at the two-leaf stage of growth. Control trees were not graft-inoculated. Trees were read after 12 mo of incubation.

[g] Four collections grafted onto two indicator trees each.

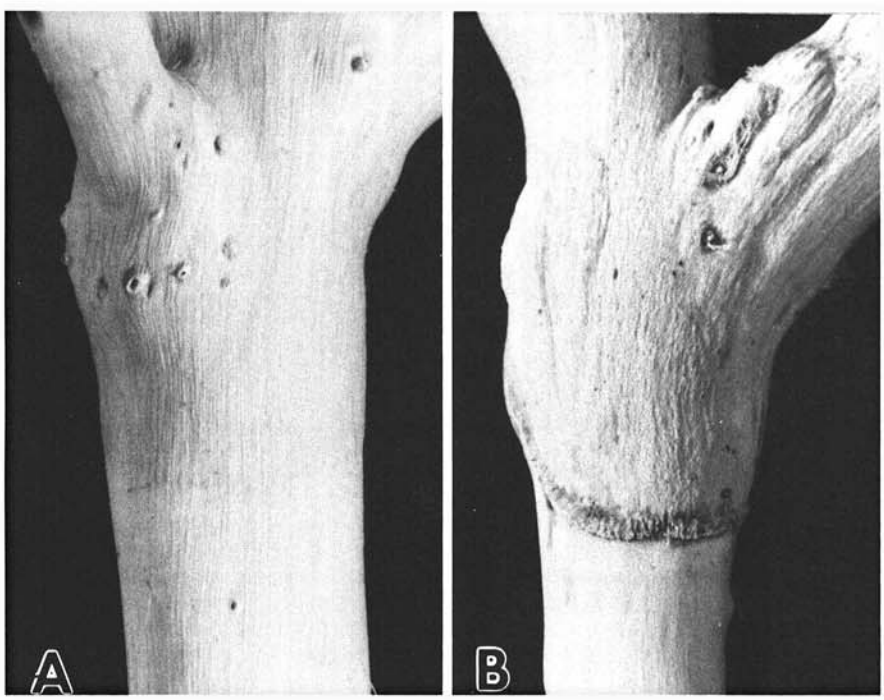


**Fig. 2. (A)** A healthy union of a young Bing sweet cherry/mahaleb tree. **(B)** Symptoms of union aberration on a young Bing sweet cherry/mahaleb tree; symptoms were evident after a 12-mo incubation.

is systemic in mahaleb (7), all budling shoots on a common rootstock should have eventually developed union symptoms if at least one shoot was diseased.

This is the first description of X-disease infection of sweet cherry trees on Colt rootstock. With the exception of the reddish-cast leaves (presumably the initial disease symptom), the chronic infection (symptoms expressed after 2 yr) is similar to that noted for trees on mazzard and Stockton morello rootstocks. Infected trees on these three rootstocks do not develop union aberration.

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**LITERATURE CITED**

1. Gilmer, R. M., and Blodgett, E. C. 1976. X-disease. Pages 145-155 in: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U.S. Dep. Agric. Agric. Handb. 437.
2. Gilmer, R. M., Moore, J. D., and Keitt, G. W. 1954. X-disease virus: I. Host range and pathogenesis in chokecherry. Phytopathology 44:180-185.
3. Granett, A. L., and Gilmer, R. M. 1971. Mycoplasmas associated with X-disease in various *Prunus* species. Phytopathology 61:1036-1037.
4. Hoy, J. W., and Mircetich, S. M. 1984. Prune brownline disease: Susceptibility of prune rootstocks and tomato ringspot virus detection. Phytopathology 74:272-276.
5. Kirkpatrick, B. C., Stenger, B. C., Morris, T. J., and Purcell, A. H. 1987. Cloning and detection of DNA from a nonculturable plant pathogenic mycoplasma-like organism. Science 238:197-200.
6. Mircetich, S. M., and Civerolo, E. L. 1972. Relationship between stem pitting in peach and other *Prunus* species. Phytopathology 62:1294-1303.
7. Mircetich, S. M., Moller, W. J., and Nyland, G. 1977. Prunus stem pitting in sweet cherry and other stone fruit trees in California. Plant Dis. Rep. 61:931-935.
8. Powell, C. A. 1984. Comparison of enzyme-linked immunosorbent assay procedures for detection of tomato ringspot virus in woody and herbaceous hosts. Plant Dis. 68:908-909.
9. Rawlins, T. E., and Thomas, H. E. 1941. The buckskin disease of cherry and other stone fruits. Phytopathology 31:916-925.
10. Rosenberger, D. A., Cummins, J. N., and Gonsalves, D. 1989. Evidence that tomato ringspot virus causes apple union necrosis and decline: Symptom development in inoculated apple trees. Plant Dis. 73:262-265.
11. Schneider, H. 1945. Anatomy of buckskin-diseased peach and cherry. Phytopathology 35:610-635.
12. Smith, S. H., Stouffer, R. F., and Soulen, D. M. 1973. Induction of stem pitting in peaches by mechanical inoculation with tomato ringspot virus. Phytopathology 63:1404-1406.
13. Thomson, S. V. 1982. Sweet Ann and Utah Giant sweet cherries introduced. Fruit Var. J. 36:59-60.
14. Uyemoto, J. K. 1975. A severe outbreak of virus-induced grapevine decline in Cascade grapevines in New York. Plant Dis. Rep. 59:98-101.
15. Uyemoto, J. K., Luhn, C. F., Asai, A., Beede, R., Beutel, J. A., and Fenton, R. 1989. Incidence of ilarviruses in young peach trees in California. Plant Dis. 73:217-220.