

Apple Mosaic Virus in U.S. Filbert Germ Plasm

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ABSTRACT

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Apple mosaic virus (ApMV) has been reported to cause a mosaic disease of filberts (*Corylus avellana*). In this study, seven ApMV-infected filbert cultivars were detected by enzyme-linked immunosorbent assay (ELISA) in the U.S. filbert germ plasm collection at Corvallis, OR. All plants tested negative by ELISA for prunus necrotic ringspot virus. ApMV-infected trees may be symptomless or may exhibit chlorotic ringspots and line patterns. This is the only virus disease of filberts reported in the United States.

A mosaic disease of filberts (*Corylus avellana* L.) has been known since 1957 in Italy, where it is widespread (11). Filbert mosaic has also been reported in France (8) and Great Britain (12). Cardin and Marenaud (4) detected a virus serologically related to tulare apple mosaic from apple trees that had been inoculated with mosaic-infected filbert tissue. Apple mosaic virus (ApMV) was detected by enzyme-linked immunosorbent assay (ELISA) in filbert trees with ringspots and line patterns, and mycoplasma-like organisms were found in trees with yellows and dieback (11). Barba and Quacquarelli (1) associated ApMV with filbert trees showing line patterns and prunus necrotic ringspot virus (PNRV) with ringspot symptoms. ApMV is known to cause chlorotic lines and rings in many rosaceous species but in very few hosts outside the Rosaceae. Hop (*Humulus lupulus*), birch (*Betula* sp.), and horse chestnut (*Aesculus hippocastanum*) are nonrosaceous hosts in which ApMV has been reported to occur in nature (6,10,12).

A ring-pattern virus disease of filbert was first observed in the United States in 1972, in Corvallis, OR. Leaf symptoms were observed at an Oregon State University (OSU) field planting on the cultivar Tonda Rossa. The infected plant had been imported from Italy (2) and was subsequently destroyed. ApMV has since been detected in the OSU filbert breeding material in Corvallis (3).

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The U.S. Department of Agriculture operates the National Clonal Germplasm Repository (NCGR) in Corvallis that houses the U.S. filbert germ plasm collection. Clonal filbert germ plasm is maintained as field-grown plants on the grounds of the OSU Horticulture Research Farm in Corvallis. There are 221 *Corylus* accessions representing both cultivars and wild species. In May 1986, chlorotic line patterns and ringspots similar to those associated with ApMV were observed on several filbert cultivars in the NCGR collection. Serological testing was undertaken to determine the incidence of ApMV and PNRV in the filbert germ plasm collection.

MATERIALS AND METHODS

Antisera against ApMV (PVAS 254) and PNRV strain G (PVAS 22) were obtained from the American Type Culture Collection, Rockville, MD. Young leaves were collected in May 1986, from each *Corylus* accession and tested for ApMV by standard ELISA (5). Plants were similarly tested for PNRV and retested for ApMV in March 1987.

Leaves were ground with a mortar and pestle in phosphate-buffered saline

(PBS), 0.02 M phosphate + 0.15 M NaCl with 0.05% Tween 20, 2% polyvinylpyrrolidone (mol wt 10,000), and 0.02% ovalbumin. Aliquots of 200 μ l were added to polystyrene microplates (Immulon II, Dynatech Labs. Inc., Alexandria, VA) that had been coated with 2 μ l/ml IgG for ApMV or 1 μ l/ml IgG for PNRV. Virus particles were labeled with alkaline phosphatase-conjugated IgG at 1:500 for ApMV and at 1:800 for PNRV. *p*-Nitrophenyl phosphate substrate solution was allowed to react for 60 min, and absorbance at 405 nm was recorded with a Biotek model 308 microplate reader (Biotek Instruments Inc., Burlington, VT).

RESULTS

Eight *Corylus* accessions tested positive for ApMV, and 136 accessions tested negative. All *Corylus* accessions tested negative for PNRV. Known sources of PNRV strain A and strain G produced absorbance values greater than 10 times the background on the PNRV plates and were indistinguishable from healthy controls on the ApMV plates. All accessions that had been observed with mosaic symptoms tested positive for ApMV. In addition, several symptomless trees tested positive (Table 1). Symptoms consisted of chlorotic ringspots and line patterns on the older foliage. By early summer, this older foliage was hidden by new symptomless growth. Symptoms were sometimes expressed on a single branch or on one side of the tree, but the virus was detected throughout the tree. The virus was more readily detected from new symptomless foliage than from diseased leaves.

Table 1. NCGR *Corylus* accessions infected with apple mosaic virus (ApMV)

Accession number	Cultivar	Absorbance ^a (405 nm)	Symptoms	Origin
...	Healthy control	0.059	No	Oregon
9001	ApMV-infected control	1.707	Yes	Oregon
56	Badem	2.952	No	Turkey
75	Grossal de Constanti	2.947	Yes	Spain
51	Mortarella	1.458	Yes	Italy
29	Pallaz	1.925	Yes	Turkey
32	Sivri Ghiagli	2.441	Yes	Turkey
112	Sivri Ghiagli	0.969	Yes	Turkey
43	Tombul	2.824	No	Turkey
21	Tonda Bianca	2.563	Yes	Italy

^a Mean of two replicates.

The OSU trees from which infected accessions were propagated were also tested for ApMV and PNRV. Additional OSU trees were tested as well. All of the OSU trees that were sources for infected NCGR accessions tested positive for ApMV. In addition, the cultivars Negret, Pautet, San Joan, Ribet, and Extra Ghiaghli tested positive in the OSU planting. Nearly all of these infected trees were symptomless.

DISCUSSION

All of the NCGR filbert accessions infected with ApMV were infected before their arrival at NCGR. It seems likely that the virus was introduced when these plants were imported from Europe; however, additional trees may have become infected naturally while growing at the OSU research planting. A low percentage of seed transmission of ApMV has been demonstrated in *Corylus* (3). The virus was transmitted to seedlings when infected Negret trees were used as either the male or female parent. It is not known, however, whether a healthy tree is able to become infected via pollen. The virus has been detected in both the embryo and endosperm of unripe horse chestnut seed (*Aesculus hippocastanum*) but not in mature seed or seedlings (12).

All of the mosaic symptoms observed on filbert trees in Corvallis appear to be caused by ApMV. Barba and Quacquarelli detected PNRV in trees with ring-pattern symptoms (1); however, trees with these same symptoms tested negative for PNRV in this study. PNRV is the only other ilarvirus that is reported to be serologically related to ApMV (7). McMorran and Cameron, however, found no evidence of any antigen/antiserum reaction between the ApMV and NRSV

serogroups by ELISA (9). They failed to detect 10 isolates of ApMV, including the filbert isolate, using six NRSV antisera. They also failed to detect 26 NRSV isolates using three ApMV antisera.

Field spread of ApMV has been observed in filberts in Italy. The percentage of infected trees in an orchard increased from 1 to 4.5 over a 6-yr period (11). No vector was associated with this spread. Natural spread is unknown in apple except possibly by root grafts. The virus is typically distributed through infected scions or clonal rootstocks (10).

ApMV represents the first virus of significance to filberts growing in the United States. There have been no other diseases of *Corylus* in the United States attributed to a virus infection; therefore, there is no virus certification program for this crop. Whereas all of the other clonal germ plasm collections at the NCGR are grown in insect-proof screenhouses to prevent virus spread, the *Corylus* collection is maintained only as a field planting. All infected filbert trees have been removed from the NCGR field plantings and placed in isolation to prevent possible spread to adjacent filbert germ plasm. Virus elimination procedures will be attempted with these infected cultivars. In apples, ApMV does not become fully systemic. Mosaic-free apple trees can be readily propagated from actively growing shoot tips, especially after heat treatment (10).

Continued ELISA will be needed to determine if virus spread is occurring. Additional research will be required to determine whether infected pollen poses a threat to healthy trees. We do not know if this virus occurs in any commercial filbert orchards or nurseries. Mosaic symptoms have not been reported outside these research plantings, thus it is

imperative to eliminate this inoculum source. All future introductions of *Corylus* germ plasm to the United States should be tested for ApMV before they are released.

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