

SHORT COMMUNICATION
NATURAL WEED HOSTS OF *APPLE MOSAIC VIRUS* IN HAZELNUT
ORCHARDS IN TURKEY

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SUMMARY

A total of 70 weed samples from 31 plant species belonging to 16 families (*Apiaceae*, *Araceae*, *Araliaceae*, *Asteraceae*, *Campanulaceae*, *Convolvulaceae*, *Dryopteridaceae*, *Euphorbiaceae*, *Geraniaceae*, *Lamiaceae*, *Plantaginaceae*, *Ranunculaceae*, *Rosaceae*, *Rubiaceae*, *Scrophulariaceae* and *Urticaceae*) were collected in orchards of hazelnut (*Corylus avellana* L.) in Samsun and Ordu provinces of Turkey and tested by DAS-ELISA and biological assay methods. Samples belonging to *Scandix* sp., *Artemisia vulgaris* L., *Campanula* sp., *Galeopsis* sp., *Salvia verbenaca* L., *Prunella* sp., *Clematis vitalba* L. and *Rubus canescens* L. were found to be infected by *Apple mosaic virus* (ApMV). This is the first report of ApMV infection of these plants under natural conditions. The epidemiology of ApMV in hazelnut orchards is discussed.

Key words: ApMV, hazelnut, weed host.

Turkey has the largest production of hazelnut (*Corylus avellana* L.) in the world, followed by Italy, the USA and Spain (Anonymous, 2004). Hazelnut is grown extensively in the Black Sea Region with nearly 33% of the production in Samsun and Ordu provinces (Anonymous, 2003), which are located along the central coast of the Black Sea. 'Palaz', 'Tombul' and 'Sivri' are the main cultivars and are grown as bush-like trees called 'ocak' in the region.

Apple mosaic virus (ApMV; genus, *Ilarvirus*, family *Bromoviridae*) is one of the most important pathogens of hazelnut and may have a synergistic effect when co-infecting with *Prunus necrotic ring spot virus* (PNRSV; genus, *Ilarvirus*; family, *Bromoviridae*) (Anonymous, 2004). ApMV infection in hazelnut has been reported in many countries, including Italy (Scaramuzzi and Ciferri, 1957), France (Marenaud and Germain, 1975), Spain (Tasias, 1975), the UK (Sweet and Barbara, 1979) and

the USA (Postman and Cameron, 1987). Previously, ApMV had been detected in 15% of the 34 hazelnut clones imported into the USA from Turkey (Postman and Mehlenbacher, 1994). In a previous study of 62 orchards in four districts of Samsun province done in April-May 2002-2003, it was shown that the percentage of infected hazelnut samples determined by ELISA varied between 10 and 18.3% (Arli-Sokmen *et al.*, 2004).

ApMV infection in hazelnut in Bartin, Duzce and Zonguldak provinces in the western Black Sea Region of Turkey was also reported recently by Akbas *et al.* (2004).

ApMV can infect, either experimentally or naturally, over 65 species in 19 different families (Fulton, 1972). Natural hosts of ApMV include apple, rose, hazelnut, horse chestnut, raspberry, birch, hops (Rybicki, 1995), stone fruits (Myrta *et al.*, 2003) and strawberry (Tzane-takis and Martin, 2005). Many ApMV isolates are serologically distinct and induce a range of symptoms in herbaceous test plants. Some isolates are serologically related to PNRSV (Fulton, 1972; Casper, 1973). Usually, ApMV can be transmitted, although with difficulty, by mechanical inoculation of sap from woody plants to several herbaceous species (Fulton, 1972; Shiel and Berger, 2000). The natural vector of ApMV is unknown (Postman and Mehlenbacher, 1994), however, ApMV has been detected in seed and pollen of hazelnut (Aramburu and Rovira, 2000).

The objective of this work was to test for ApMV in weeds from hazelnut orchards in Samsun and Ordu provinces by using ELISA and sap inoculation tests. Surveys were conducted between May and June 2003-2004 in a total of 12 commercial hazelnut orchards in Central, Terme, Carsamba, Ondokuz and Mayis districts of Samsun province, and Unye and Fatsa districts of Ordu province. Weed samples were collected from around the bases of hazelnut bushes showing symptoms characteristic of infection by ApMV (yellow bands and spots, oak leaf pattern). Most of the weed species tested were symptomless, but some of the weeds sampled showed virus-like symptoms (Table 1).

Each plant sample was identified, put in a polyethylene bag and kept at -20°C until ELISA and biological tests were completed within 4 months. DAS-ELISA was

performed according to Clark and Adams (1977) using ApMV polyclonal antiserum (hop isolate) from Loewe (Sauerlach, Germany) at the recommended dilution. Samples or negative and positive controls (Loewe, Sauerlach, Germany) were ground (1 g sample per 10 ml buffer) in extraction buffer (PBS: 0.13 M NaCl, 0.014 M KH_2PO_4 , 0.08 M $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.002 M KCl, pH: 7.4) containing 0.05% Tween-20, 1% polyvinyl pyrrolidone and 1% skimmed milk powder. Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grödigg/Salzburg, Austria). Samples were considered positive when the absorbance values at 405 nm (A_{405}) exceeded the mean of the negative control (negative control of Loewe) by a factor of at least three (Aramburu and Rovira, 1998).

In a preliminary study, *Cucumis sativus* L., *Vigna unguiculata* (L.) Walp, *Phaseolus vulgaris* L., *Petunia hybrida pendula* Vilm., *Amaranthus retroflexus* L., *Gomphrena globosa* L., *Nicotiana tabacum* L. "Xanthi nc" or *Nicotiana clevalandii* L. were inoculated with ApMV hazelnut isolate. Only *P. vulgaris* (PI 212110, the collection of Western Regional Introduction Station, Washington, the USA) produced symptoms. These were sys-

temic chlorosis and necrotic lines on upper leaves. Therefore, this species was used for virus isolation and maintenance. Extracts from weed samples that gave high absorbance values in ELISA were inoculated mechanically onto bean leaves at the 2-4 leaf stage (four replicates for each species) after homogenization in 0.03 M phosphate buffer (pH: 8.0) including 2% 2-mercaptoethanol (Fulton, 1972). After rinsing with tap water, plants were maintained in a growth cabinet at 20-22°C. Inoculated plants were tested for the presence of ApMV by ELISA to confirm the results of previous serological testing.

A total of 70 weed samples from 31 plant species belonging to *Apiaceae*, *Araceae*, *Araliaceae*, *Asteraceae*, *Campanulaceae*, *Convolvulaceae*, *Dryopteridaceae*, *Euphorbiaceae*, *Geraniaceae*, *Lamiaceae*, *Plantaginaceae*, *Ranunculaceae*, *Rosaceae*, *Rubiaceae*, *Scrophulariaceae* or *Urticaceae* were collected and tested by DAS-ELISA.

ApMV was detected by DAS-ELISA in 1 sample of *Scandix* sp., 1 sample of *Artemisia vulgaris* L., 1 sample of *Campanula* sp., 1 sample of *Galeopsis* sp., 1 sample of *Salvia verbenaca* L., 2 samples of *Prunella* sp., 1 sample of *Clematis vitalba* L. and 5 samples of *Rubus canescens*

Table 1. Assays of weed species found to be positive for ApMV.

Species (Family)	Life Form	Symptom on weed leaf	Symptom on bean leaf	A405 values	
				2 hours ^a	16 hours ^b
<i>Scandix</i> sp. (<i>Apiaceae</i>)	Annual	-	+	0.13	0.79
<i>Artemisia vulgaris</i> L. (<i>Asteraceae</i>)	Perennial	-	+	0.12	1.18
<i>Campanula</i> sp. (<i>Campanulaceae</i>)	Annual	-	+	0.13	0.93
		-	-	0.08	0.19
		-	-	0.10	0.22
		-	-	0.14	0.88
<i>Salvia verbenaca</i> L. (<i>Lamiaceae</i>)	Perennial	Chlorosis	+	0.14	0.88
<i>Galeopsis</i> sp. (<i>Lamiaceae</i>)	Annual	-	+	0.15	0.60
		-	-	0.08	0.18
		-	+	0.17	1.37
<i>Prunella</i> sp. (<i>Lamiaceae</i>)	Annual	Oak leaf pattern	+	0.21	2.33
		-	-	0.09	0.22
		-	+	0.21	2.42
		-	+	0.19	1.07
<i>Clematis vitalba</i> L. (<i>Ranunculaceae</i>)	Perennial	Yellow flecking	+	0.21	0.86
		Yellow flecking	+	0.23	2.73
		Yellow flecking	+	0.16	0.69
		Yellow flecking	+	0.20	0.56
		-	-	0.10	0.24
<i>Rubus canescens</i> L. (<i>Rosaceae</i>)	Perennial	-	-	0.09	0.22
		-	-	0.09	0.24
		-	-	0.08	0.12
		-	-	0.08	0.16
Negative Control (Loewe)				1.78	3.40
Negative Control (Buffer)					
Positive Control (Loewe)					

- : Symptomless; ^a : absorbance values for 2 h substrate incubation; ^b : absorbance values for 16 h substrate incubation. + : ApMV-type symptom.

L. (Table 1). Bean plants mechanically inoculated with extracts of ELISA-positive plants showed systemic chlorosis and necrotic lines on non-inoculated distant apical leaves two to five weeks after inoculation. Symptoms on bean leaves were almost identical to those induced by infection with ApMV-hazelnut isolate.

In addition to these symptoms, bean leaves inoculated with the sap of *Campanula* sp. and *Prunella* sp. showed stunting and vein clearing symptoms, respectively. All inoculated bean plants were tested by DAS-ELISA at two different times to confirm the presence of ApMV in symptomatic plants. The first test was performed at three weeks after inoculation, and only two bean plants inoculated with extracts from *R. canescens* and one plant inoculated with extracts from *Prunella* sp. were positive by ELISA. Six weeks after inoculation, all plants inoculated with extracts from ELISA-positive weed plants tested positive for ApMV in ELISA tests. None of the plant species, *Arum italicum* Mill., *Hedera helix* L., *Carduus* sp., *Galinsoga* sp., *Lapsana communis* L., *Convolvulus arvensis* L., *Calystegia sepium* L., *Dryopteris* sp., *Euphorbia populus* L., *Geranium dissectum* L., *Salvia virgata* L., *Stachys* sp., *Melissa officinalis* L., *Plantago lanceolata* L., *Ranunculus repens* L., *Rosa* sp., *Fragaria* sp., *Rubus* sp., *Galium aparine* L., *Veronica polita* L., *Veronica* sp., *Urtica dioica* L., *U. urens* L., was found to be infected.

R. canescens is a very widespread weed species in hazelnut plantations in the middle Black Sea region of Turkey (Mennan *et al.*, 1999). However, it is also an important species because of its edible fruits and medicinal value throughout Turkey (Dogan *et al.*, 2004). Most of *R. canescens* plants that were found to be ApMV-positive showed symptoms of yellow flecking in our study. In contrast, ApMV-infected *Rubus* plants in North America were symptomless, but some *R. idaeus* plants in Germany exhibited apparent line patterns and/or yellow speckling (Baumann *et al.*, 1982). ApMV has been detected in *R. idaeus*, *R. occidentalis* and *R. ursinus* in Canada and the USA (Converse and Casper, 1975; Baumann *et al.*, 1982). No information is available on seed or pollen transmission of ApMV in *Rubus*. Other weed species displaying virus-like symptom in our study are shown in Table 1.

Color development in the ELISA tests with the weed samples and even with the infected bean leaves that had been inoculated by using the sap of ApMV infected-hazelnut leaf was very slow in our study. The results of ELISA shown in Table 1 were taken after overnight substrate incubation. One reason for the low ELISA values could be that the ApMV isolates infecting weed species in the region may be serologically distantly related to the hop isolate that was used for development of the ApMV antiserum used in this study (Loewe Biochemica, Sauerlach, Germany). The positive control supplied by the Loewe Biochemica usually gave satisfac-

tory results at 2 h and 16 h substrate incubations (Table 1). ApMV is reported to have several serological strains (Fulton, 1972; Casper, 1973). A further study is required to search for the presence of ApMV in weeds and other crop plants in more detail by using mixture of monoclonal antibodies specific for different ApMV isolates or variants. Other reasons for the slow development of color in ELISA tests could be low virus titres and/or uneven distributions of virus in host tissues, both features of infections by ilarviruses (Gruntzig *et al.*, 1994). Sano *et al.* (1985) also reported weak reactions of inoculated plants against ApMV antiserum in work with the hop isolate from Japan.

Weeds are abundant in hazelnut orchards because of poor weed control by growers in the region. Transmission of ApMV from woody host to herbaceous plants is usually difficult by mechanical means (Shiel and Berger, 2000). Our study did not include experiments to elucidate the mechanism of ApMV transmission to weeds in nature. However, it was reported by Hunter *et al.* (1958) that root grafts may cause the spread of ApMV when young trees are closely spaced in a nursery (Fulton, 1981). Weed species collected during surveys were growing very close to the hazelnut bushes (ocak), which had ApMV-type symptoms in our study. Hazelnut is reproduced by clones in Turkey, and each ocak has 5-10 stems roots of which can spread horizontally for about a meter (Dr. N. Beyhan, personal communication). An explanation for ApMV infection of weeds could be that they may have become infected through root grafting between hazelnut and weed. Some viruses have been transmitted between woody and herbaceous species by various forms of grafting, even though the species were unrelated. It was not clear if this transmission occurs because of prolonged contact or organic connections between these species. However, it was assumed that this type of grafting would not form a functional graft union (Fulton, 1966).

Thrips are thought to be involved in the transmission of PNRSV (Greber *et al.*, 1992) and Tobacco streak virus (Kaiser *et al.*, 1982), both ilarviruses. Also, phylogenetic analysis of sequences of the protein products encoded by ApMV RNA 1 and 2 and the putative movement protein encoded by RNA 3 suggested that ApMV is more closely related to Alfalfa mosaic virus (AMV; genus *Alfamovirus*, family *Bromoviridae*) than to other ilarviruses, and it has been suggested that AMV should be placed in the genus *Iilarvirus* (Shiel and Berger, 2000). AMV is non-persistently transmitted by 14 species of aphids (Jaspars and Bos, 1980). Another explanation for ApMV infection of weeds could be that ApMV may have an unidentified arthropod vector. However, in hazelnut orchards throughout the region, a slow natural spread of ApMV infection was observed. This may indicate that ApMV is spread by a slow-moving arthropod vector.

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