SHORT COMMUNICATION

INCIDENCE AND NATURAL SPREAD OF *APPLE MOSAIC VIRUS* ON HAZELNUT IN THE WEST BLACK SEA COAST OF TURKEY AND ITS EFFECT ON YIELD

B. Akbaş and K. Değirmenci

Plant Protection Central Research Institute, 06172 Ankara, Turkey

SUMMARY

The status of *Apple mosaic virus* (ApMV), causal agent of hazelnut mosaic, was investigated in Turkey by examining hazelnut plants visually, serologically and by biological indexing. A survey of 213 orchards was carried out in 2005-2008 during the growing season in the west Black Sea region. A total of 1465 hazelnuts were sampled and tested. Disease symptoms were observed in 84 orchards. ELISA and transmission to *Chenopodium quinoa* and *Cucumis sativus* confirmed the presence of ApMV in symptomatic samples only. Average infection rate was 13.6%. The effect of infection on hazelnut trees were examined in three different provinces collecting data on plant yield from selected trees. Crop losses ranged from 19.7 to 35.6% (28.2% in average) and infected trees showed a slight growth reduction.

Key words: Hazelnut mosaic, ApMV, disease incidence, diagnosis, ELISA.

Apple mosaic virus (ApMV) infects a wide natural range of woody plants, e.g. apple, almond, apricot, birch, blackberry, hazelnut, hops, horse chestnut, raspberry, red currant, rose, strawberry and sweet cherry. The virus has no vector (Rybicki, 1995), does not seem to spread naturally (Fulton, 1981; Howell *et al.*, 1990), is not transmitted by dodder (Fulton, 1972; Eppler, 1992) and is not pollen- or seed-borne. However, ApMV is readily graft-transmissible and persists in propagative material (budwood and grafted nursery plants) which is probably the main source of infection (Petrzik, 2005). Spread through soil in nurseries via root graft has been observed (Hunter *et al.*, 1958; Dhingra, 1972), and virus presence has recently been identified in naturally infected weeds in hazelnut orchards (Arlı-Sökmen *et al.*, 2005). ApMV is known to cause a mosaic disease of hazelnut (*Corylus avellana* L.) in several European countries and the USA (Ragozzino, 1980; Postman and Cameron, 1987; Rovira and Aramburu, 1998; Aramburu and Rovira, 2000; Postman, 2002; Piskornik *et al.*, 2002; Kobylko *et al.*, 2005). Crop losses of up to 42% were recorded (Aramburu and Rovira, 1995).

Hazelnut is one of the native plants of Turkey, which has a significant position among the other hazelnut producing countries in the world. The production in the west Black Sea coast includes the provinces of Bartın (3,753 ha), Düzce (40,186 ha), Kocaeli (9,106 ha), Sakarya (25,527 ha) and Zonguldak (23,563 ha), encompassing approximately 20% of cultivated area (Anonymous, 2006).

The sanitary condition of the Turkish hazelnut industry with reference to mosaic has been known for many years, as shown by published reports (Postman and Mehlenbacher, 1994). However, only in more recent times the importance of mosaic as one of the most harmful diseases of the industry was realized (Akbaş *et al.*, 2004; Arlı-Sökmen *et al.*, 2004).

Surveys of Turkish hazelnut orchards for detection of viruses were initiated in 2002 (Akbaş *et al.*, 2004) and were extended in 2005-2008, as reported in the present paper. In this latter survey the prevalence and incidence of ApMV in hazelnut orchards of the west Black Sea coast of Turkey were determined. To this aim, the major hazelnut-growing areas in the region were inspected systematically. Leaves were collected at random from each quadrant of a "shrub". Hazelnut is traditionally cultivated as a bush like-trees in Turkey, and each shrub includes 4 or more trees. Thus, tissues from the main scaffold limbs were combined and extracted for ELISA testing. A total of 1465 shrubs were sampled in 213 orchards from the five hazelnut-growing provinces (Fig. 1). Thirteen, 78, 20, 53 and 49 orchards were surveyed in Bartın, Düzce, Kocaeli, Sakarya and Zonguldak, which resulted in 97, 492, 144, 371 and 361 samples, respectively. Samples were collected from 2- to 10-year-old trees. Observations on symptom expression were made in spring and summer. DAS-ELISA was done with a commercial (Agdia, USA) using the manufacture's protocol. A sample was scored positive if both wells had an OD greater than triple the negative control. Confirmatory tests were done by mechanical inoculation onto *Chenopodium quinoa* and *Cucumis sativus*.

Corresponding author: B. Akbaş
Fax: +90.312.31515 31
E-mail: birol_akbas@zmmae.gov.tr

Fig. 1. Surveyed areas of the west Black Sea coast of Turkey.

The effect of ApMV infection on the performance of hazelnut trees was assessed measuring fresh weight of shrub yield and its vigour. Virus-negative and virus-positive shrubs were predetermined serologically. Selected shrubs in an orchard were in the same rows, had the same age and belonged to the same variety. Selected orchards were four in the province of Bartın, six in Düzce, and six in Zonguldak. Orchards were established on the plain in Düzce, and on the hills in Bartın and Zonguldak. Two or three ApMV-positive and negative shrubs from every orchard surveyed were identified and yield data were assessed for three years. Data taken from all shrubs were evaluated for each orchard separately. Plant growth was also observed.

All data were subjected to analysis of variance (ANOVA) using SPSS procedures and the means were compared using Duncan (1955) multiple range test. Abbott's formula was used to determine % influence of ApMV on hazelnut yield.

ApMV infections were observed in almost half the surveyed orchards with incidence ranging from 4 to 20%. Symptoms consisting of yellow rings and lines, yellow flecking, oak leaf pattern and broad vein banding (Fig. 2) were generally expressed on a single branch or on one side of the tree. ApMV was detected throughout each infected tree, but not necessarily in all trees of the same shrubs (each shrub includes 4 or more trees). In general, symptomatic trees occurred in close proximity to one another. Taking into account the effects of regions, grower practices and orchard ages, disease incidence did not vary significantly during the four years of observations.

Fig. 2. ApMV symptoms on hazelnut leaves.

A total 13,6% of the ELISA-tested hazelnut trees (197 out of 1465 shrubs) were infected by ApMV. All trees with mosaic symptoms tested positive, whereas most of the symptomless trees reacted negatively. The highest extinction values were recorded from young leaves collected in spring. Even so, ApMV was detected from diseased leaves also in summer.

Transmission of ApMV to *C. quinoa* and *C. sativus* was obtained under greenhouse conditions. *C. quinoa* reacted with mottling, whereas *C. sativus* showed chlorotic local lesions followed by systemic yellowing and stunting. These plants tested positive for ApMV in ELISA. ApMV was detected in 84 of 213 orchards. These numbered 5, 33, 8, 22 and 21 in Bartın, Düzce, Kocaeli, Sakarya and Zonguldak provinces, respectively. The highest ApMV infection was found in Düzce (14.8%) and the lowest in Kocaeli (12,5 %) (Table 1).

The assessment revealed a marked influence on yield and growth of ApMV in infected shrubs. Mosaic symptoms were especially prominent in trees older than 6 years. As shown in Table 2, in half of the orchards (8 out of 16) there was a significant variation in nut yields between virus-infected and healthy shrubs in function of the year. However, in the other half, differences were significant in function of the sanitary status (healthy vs infected shrubs) rather than the year (Table 3). Nut cluster weight was reduced in average by 28.18% in ApMV-infected hazelnuts, nut size was also reduced and many clusters contained empty nuts. Significant differences in yield losses were recorded among provinces and orchard topography (hilly vs. plain). Decrease in fruit yield of ApMV-infected shrubs averaged 19.66% in Bartın, 35.61% in Düzce and 28.27% in Zonguldak (Table 4). A slight growth reduction, as estimated visually, was observed in infected shrubs, which was more prominent in those older that 6 years. To our knowledge, this is the first extensive sanitary assessment of hazelnut mosaic in Turkey. As compared with the incidence of ApMV on hazelnut reported in the first virological study of Turkish hazelnut (Akbaş *et al*., 2004), the percentage of infected trees currently detected was much lower (13.58% vs. 73.3%). This difference is due to selection of samples. In our previous study, samples were selected taking symptoms into account with the aim of determining virus presence. This figure (73.3%)

Table 1. ApMV incidence in hazelnut orchards of the west Black Sea coast.

Provinces	Tested orchards (No.)	Infected orchards (No.)	Tested shrubs (No.)	Infected shrubs (No.)	Mean infection rate (%)
Bartın	13	5	97	13	13.4
Düzce	78	33	492	73	14.83
Kocaeli	20	7	144	18	12.5
Sakarya	53	20	371	47	12.66
Zonguldak	49	19	361	46	12.74
Total	213	84	1465	197	13.58

Table 2. Means of total nut cluster weight (kg) of healthy and ApMV-infected hazelnut shrubs in three harvest years.

Ochards	1st year		2nd year		3rd year		P value
	Healthy	Infected	Healthy	Infected	Healthy	Infected	
Bartın 1	17.1±0,.21 a	16.0 ±0.23b	17.56 ± 0.40 a	15.2±0.12 c	15.96±0.12 b	15.4±0.20 c	0.004
Bartın 2	9.90±0.057 a	8.66±0.29 c	9.73 ±0.033 ab	7.66±0.26 d	9.06±0.29 bc	7.36±0.27 d	0,05
Düzce 1	7.1±0.057 b	3.76±0.28c	9.0±0.11 a	3.46±0.26 cd	8.93±0.12 a	2.76±0.14 c	0.004
Düzce 2	5.33±0.088 b	4.06±0.66bc	9.9±0.31 a	4.0±0.28 bc	5.23±0.14 b	4.90±0.056 b	0.003
Zonguldak1	7.16±0.23 b	4.33±0.16d	7.16±0.23 b	5.63±0.21 c	10.60±0.37 a	4.36±0.26 d	0.0005
Zonguldak2	7.03±0,26 a	3.43±0,23 c	6.30±0.057 ab	4.13±0.20 c	5.80±0.11 b	3.50±0.25 c	0.05
Zonguldak3	3.96±0,14 b	3.13±0,088c	6.53±0.31 a	3.43±0.17 c	4.26±0.14 b	3.43±0.23 c	< .0001
Zonguldak4	4.10±0,057 a	3.23±0,14d	3.93±0.21 ab	3.36±0.18 cd	3.66±0.088 bc	3.20±0.11 d	0.03

* Figures followed by a small letter are significantly different (Duncan's multiple range test).

Table 3. Means of total nut cluster weight (kg) of ApMV-infected hazelnut shrubs.

Orchards	Healthy	Infected	P value
Bartın 3	13.44 ±0.37 a	4.21 ±0,24 b	0.36
Bartın 4	5.93 ±0.43 a	5.18±0.47 b	0.71
Düzce 3	8.51±0.19 a	7.19±0.21 b	0.26
Düzce 4	7.61±0.21 a	5.33±0.23 b	0.61
Düzce 5	5.85±0.19 a	4.84±0.25 b	0.09
Düzce 6	13.28±0.23 a	12.51±0.19 b	0.41
Zonguldak 5	6.16 ±0.076 a	5.63 ±0.15 b	0.33
Zonguldak 6	8.45±0.14 a	7.88±0.18 b	0.22

*Figures followed by a small letter are significantly different (Duncan's multiple range test).

Table 4. Mean crop losses in ApMV infected shrubs in the provinces surveyed.

Provinces	Minimum crop loss (%)	Maximum crop loss (%)	Mean crop loss (%)	Standard error
Bartın	3.51	68.68	19.66	7.2959
Düzce	5.80	69.09	35.61	8.1266
Zonguldak	6.75	58.00	28.27	4.4398
General mean (%)	3.51	69.09	28.18	3.6516

was the rate of positives out of total samples suspected to be infected. In the current survey, samples were collected systematically, the survey being targeted to the number of shrubs in each province. Field spread of ApMV was not apparent during our four-year study. The mechanism of ApMV transmission in the field is unknown (Shiel and Berger, 2000). No natural spread was recorded by Fulton (1981) and Howell *et al.* (1990). The only effective transmission of ApMV in woody plant orchards is by infected propagative materials. This notion supports our observations on natural spread, even though we did not address the mechanism of ApMV transmission in hazelnut orchards. The low incidence of ApMV in cultivated hazelnut populations and the absence of infection in wild seedling populations and the apparent lack of spread during our study, suggest that transmission by foliar or root contact among trees in hazelnut shrubs may be pathways of virus spread in commercial orchards. In other host plants transmission of ApMV was claimed to occur by foliar contact (hops) or root grafting (apple) (Hunter *et al.,* 1958; Dhingra, 1972; Pethybridge *et al*., 2002). Arlı-Sökmen *et al*. (2005) reported that ApMV-infected weeds are common place in hazelnut plantations of the central Black Sea region and that the virus could spread via root grafting, weeds or an unidentified arthropod vector. However, citing Fulton's (1966) paper, they wrote that this type of transmission would be unlikely. Arlı-Sökmen *et al*. (2005) observed a slow natural spread of ApMV in hazelnut orchards and attributed it to a slow migrating arthropod vector. In our investigation, diseased shrubs were clustered and this was attributed to transplanting of infected rooted suckers in spaces in close proximity to shrubs from which rooted suckers were taken. Our data on reduced nut yields in ApMV-infected hazelnut trees are in agreement with those reported by Aramburu and Rovira (1995), who estimated a 42% crop loss. In our study, the heaviest crop loss was 35.6% in Düzce whereas lower losses were recorded in Bartın (19.7%) and Zonguldak (28.3%). The effects of ApMV in other crops has been reported. For example, lower bud take and growth reduction were observed in grafted apple seedlings by Rebandel *et al*. (1979) and average losses of 25% were recorded by Martelli and Savino (1997) in almonds affected by almond mosaic, a complex disease in which ApMV plays a major aetiological role. In conclusion, we have found that ApMV is one of the most common pathogens in hazelnut orchards in the west Black Sea region of Turkey. Discussion with growers indicated that they had very little knowledge of virus diseases and their spread. The Hazelnut Improvement Program should therefore be a valuable aid to the hazelnut industry, enabling growers to reduce the incidence of ApMV infections in their plantations. To this aim, particular attention should be paid to avoid the use of diseased rooted suckers within and among orchards and provinces.

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