# Detection of *Pear Vein Yellows Disease* caused by *Apple stem pitting virus* (ASPV) in Hatay province of Turkey

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## Abstract

*Pear vein yellows disease* (PVYD) caused by *Apple stem pitting virus* (ASPV) was studied in 9 pear orchards and 3 nurseries in Hatay province of Turkey. A survey was carried out to inspect the symptoms of PVYD on pear (*Pyrus communis*). Leaf symptoms consist of yellow vein banding, reddening and flecking along the veins that were observed during the late spring to winter (dropping leaves). ApMv, ACLSV which are important viruses of pome fruits were also investigated on pear orchards and several quince (*Pyrus cydonia*) trees in pear orchards. The shoot and leaf samples were taken randomly from inspected trees in orchards and seedlings in nurseries for ApMv, ACLSV and for ASPV in April of 2008 and 2009. A total of 20 pear samples from 15 symptomatic and 5 asymptomatic young trees of local pear cultivars ('Mustafa Bey' and 'Ankara' cvs.) and 6 from quince (unknown cultivar) were collected. All samples were detected for the presence of the viruses by ELISA. Fifteen samples (ten samples selected from symptomatic plants and 5 samples from asymptomatic trees) were also tested for the viruses by Bioassay-sap inoculation. Sap extracts were mechanically inoculated on some indicator plants (*Chenopodium amaranthicolor, C. quinoa, Cucumis sativus, Gompherena globulosa, Nicotiana benthamiana, N. glutinosa, N. occidentalis*). Although, mild symptoms including vein clearing and leaf necrosis were observed on *N. glutinosa, and N. occidentalis* test plants, in general, no symptoms associated with the investigated viruses appeared on test plants. Some samples from local pear cultivars in Hatay were found to be infected with PVYD by serological tests. ASPV was found to be present in 60% of the ELISA-tested samples in 2008. This preliminary study demonstrated that a high rate of ASPV infection was present for local pear cvs. in the province. ApMV, ACLSV infections were not tested in detected samples.

Keywords: ELISA, Ilarvirus, pear, pome fruit, test plant

## Introduction

Due to its geographical position, many horticultural crops can be grown in Turkey and the northern part of Turkey, called Black Sea Region, is one of the main genetic origins of several fruits such as apple, pear, sweet cherry, walnut, hazelnut and chestnut.

The main viruses affecting pome fruit trees are *Apple stem pitting foveavirus* (ASPV), *Apple stem grooving capillovirus* (ASGV), *Apple chlorotic leaf spot tricovirus* (ACLSV) and *Apple mosaic ilarvirus* (ApMV). These viruses occur frequently in mixed infections and cause significant yield reduction (Posnette et al., 1963 and Desvignes, 1999). ASGV is a member of the genus *Capillovirus* and is widespread in rosaceous fruit trees, particularly species of *Malus* and *Pyrus*. ApMV induces bright yellow patterns on leaves, while ASPV, ASGV and ACLSV are associated with latent infections (Németh, 1986). Different combinations of mixed infections of viruses were identified in samples of infected apple, pear and quince trees from different geographical regions in Turkey (Akbaş and İlhan, 2006; Birişik et al., 2006; Çağlayan et al., 2006; Ulubaş Serçe and Ertunç, 2006), and pear decline (PD, 16SrX-C) phytoplasma was first reported in Hatay,Turkey (Sertkaya et al., 2005).

## Material and methods

Virus-like symptoms were observed on pear during field inspections in recently established pear orchards of local cultivars in the Hatay province of Eastern Mediterranean Region of Turkey in the summer of 2007. A survey was carried out to inspect the symptoms of PVYD on pear (*Pyrus communis*) trees in the Hatay province of Turkey in 2008 and 2009. For further examination of suspicious plants, samples from 20 symptomatic pear trees (3-4 years-old) of local cultivars ('Mustafa Bey' and 'Ankara' cvs.) were collected in 9 production orchards and 3 nurseries in the early spring of 2008 and 2009. Becausemany viruses can be symptomless in pome fruits, 5 samples from asymptomatic plants and 6 quince (*Pyrus cydonia*) samples from an unknown cultivar were also randomly selected. A total of 20 pear and 6 quince samples were investigated for ACLSV, ASGV and ASPV infection by ELISA (Clark and Adams, 1977). Reagents, obtained from Bioreba AG (Switzerland) were used according to the manufacturere's double antibody sandwich protocol. Plates were coated with IgG followed by incubation with a mixture of sample and alkaline phosphatase-labelled IgG. 4-Nitrophenyl phosphate substrate was used. Negative controls (healthy apple extracts) were

included in eight wells of each ELISA plate and samples were considered positive if they reached twice the Optical density (OD) of the average of the controls. Preliminary ELISAs were carried out in April 2008 and in May 2009.

Sap extracts of fifteen samples selected from symptomatic plants were mechanically inoculated on some indicator plants, *Chenopodium amaranthicolor, C. quinoa, Cucumis sativus, Gomphrena globulosa, Nicotiana benthamiana, N. glutinosa, N. occidentalis*. Young pear leaves were homogenized in 0.1 M phosphate buffer (pH 7.2) containing 2% nicotine in a pestle and mortar, and the sap extracts inoculated onto Celite-dusted leaves of herbaceous virus indicator plants. Four plants from each of the herbaceous indicator species were mechanically inoculated with the sap of a pear sample. Inoculated test plants were kept in an insect-proof growing room at 25°C±2 and 16:8 h photoperiod (day:night) for symptom observations.

## Results and discussion

Observations of symptom expression were made during spring to autmn by repeated surveys in an attempt to associate the results of laboratory tests with field symptoms. More than 100 young trees were inspected in 9 commercial orchards and 3 nurseries. Leaf symptoms consisting of yellow vein banding, reddening and spotting or flecking along the veins and poor growth, were only observed on trees (3-4 years-old) in the orchards from spring to winter (due to leaf fall) of 2008 and spring of 2009. No obvious symptoms were seen on trees except for severe vein clearing on the leaves. Mild yellow spots were also observed on young leaves of a few pear trees (Figure 1).

**Fig. 1** Vein yellowing and yellow spot symptoms of Pear vein yellows disease caused by *Apple stem pitting foveavirus* (ASPV) on naturally infected local pear cv. 'Mustafa Bey' in Hatay.

Serological tests in the first year showed that 60% of the total samples tested were infected by ASPV. Twelve of 20 pear samples were positive for ASPV by ELISA in 2008. However, four of them gave a negative serological result for the virus in 2009. In both years, young leaves taken from vegetative buds in spring gave the best results with highest readings for ASPV in ELISA. The results of field surveys and serological assays revealed that ASPV is one of the common agents of pear disease in Hatay. The evidence of the presence of ASPV have been proved in local cultivars of pear and quince in Hatay by serological assays repeated several times in both years. A high incidence of ASPV was also reported in different studies carried out on apple by molecular methods in Turkey (Birişik et al., 2006; Çağlayan et al., 2006). According to results of Kirby et al., (2001), the IC-PCR results were confirmed but two of the 22 infected trees were negative by ELISA. ApMv and ACLSV which are known as important viruses of pome fruits, were not found in the tested samples probably due to the limited number of quince and pear samples investigated in this study. All of these samples were mechanically inoculated to a standard series of herbaceous plants. Some *N. glutinosa, N. occidentalis* test plants inoculated with extracts from symptomatic pear plants showed mild vein clearing three to four weeks after inoculation. However, these plants did not test positive for ASPV in ELISA. In general, no symptoms associated with the investigated viruses appeared on test plants up to six weeks after sap inoculation. ApMV or ACLSV were not recovered by sap transmission tests. Therefore, serological test results were confirmed with biological assays for ApMV and ACLSV. ELISA which is comonly used for virus detection is a fast and simple assay with a relatively low cost, however it becomes unreliable for the detection of woody plant viruses during the summer due to a decrease in virus concentration. In several cases it is reliable only during a short period (maximum two months after bud break in spring). Although, some laboratory methods include ELISA as rapid and cheap laboratory assay for detecting ASGV, IC-PCR was reported to be most reliable for ASGV detection compared to the slow and expensive bioassays (Fuchs,

1980; Kirby et al., 2001). In preliminary results of Paunović and Jevremović (2006), in contrast to apples, detection of ASPV was not possible in young leaves nor in the flower petals of pear isolates.

The number of orchards has increased recently in high plateaus of the region. Because of the use of propagation material taken from plants in commercial orchards which are possibly infected with common virus/es, the main virus diseases, such as PVYD, spread from infected seedlings to new plantations in the region. The use of virus-free certified material for the establishment of new plantations is the main measure to prevent the spread of these viral diseases in orchards. Further detailed investigations are necessary, since ASPV is a major latent virus of pome fruits. It will also be necessary to investigate by advanced techniques local pear cultivars and other pome fruits grown in our region for the presence of the most important viruses.

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