# Evaluation of the presence and symptomology of viruses in commercial quince orchards in Turkey

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## Abstract

Turkey is the biggest quince (*Cydonia oblonga* Mill.) producer country in the world with a production of about 120.000 tons/year. Virus diseases *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV) *Apple mosaic virus* (ApMV) and *Apple chlorotic leaf spot vius* (ACLSV) are known as viral pathogens that can affect quality and quantity of quince production. This study was carried out in the Mediterranean region of Turkey between 2006 and 2008. The study was based on a survey with symptomatological observations and the detection of viruses by DAS-ELISA and/or RT-PCR techniques. During the survey, 33 commercial orchards in five different counties were visited and 115 samples were collected and examined. Laboratory results showed that 27.82% of the samples were infected by either single or mixed infection of any tested viruses. Single infection of ASPV, ACLSV and ASGV were found in 12.17%, 5.21% and 2.60% of the samples, respectively, while mixed infections of ASPV+ASGV, ASPV+ACLSV, and ASPV+ASGV+ACLSV were in 2.60%, 3.47% and 1.73% of the samples, respectively.ApMV was not found in any tested samples.

Infected trees were marked and observed monthly during the whole vegetative period for two years. The observed symptoms were evaluated in accordance with the laboratory results. During the study; leaf mosaics, leaf deformation, fruit malformation, gummy fruit, dwarfing of the tree, bud-union abnormalities and trunk deformations were observed.

Keywords: Quince, ApMV, ACLSV, ASPV, ASGV, Turkey

## Introduction

Quince is one of the most important pome fruits in Turkey, and is produced in all regions of the country. Turkey is the biggest quince (*Cydonia oblonga*) producer in the world with a production of about 120.000 tons/year (Anonymous, 2009). Several virus diseases are known to be present in quince (Waterworth, 1989). Among those virus diseases *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV) *Apple mosaic virus* (ApMV) and *Apple chlorotic leaf spot vius* (ACLSV) are known as viral pathogens that can affect quality and quantity of quince production (Nemeth, 1986). ACLSV has been reported in quince from Turkey (Akbaş and İlhan, D.; 2008.) and Greece, and ASPV from former Yugoslavia according the research activity (Paunovic and Rankovic, 1998). This study was carried out in the Mediterranean region of Turkey between 2006 and 2008 in five different counties where quince production is high. The study was based on a survey with symptomatological observations and the detection of viruses by DAS-ELISA and multiplex RT-PCR.

## Materials and methods

Survey: The survey carried out in commercial quince orchards of Adana, Niğde, Mersin, Kahramanmaraş, and Osmaniye provinces. Sampling was done according to Bora and Karaca (1970) and the number of samples collected from orchards was based on the size of the orchards. Three samples were taken from orchards smaller than 0.5 ha, four samples from 0.5-1 ha, and six samples from orchards bigger than 1 ha. Sampling was done in spring and fall during the vegetative period collecting one year old branches from selected trees.

Observations: All quince trees in the surveyed area were checked by visual observation for any kind of virus related symptoms during the whole vegetative period for two years. The visual observation results were compared with the laboratory tests.

DAS-ELISA: DAS-ELISA was performed according to Clark and Adams, (1977). Leaf and bark tissues were used in the assay.

Multiplex RT-PCR: Total RNA was extracted from healthy and infected tissues using the Promega SV total RNA kit as described by supplier. The primers for ASPV, ACLSV and ASGV were as previously described by Menzel et al., (2003) and for ApMV by Hassan et al. (2005). The multiplex RT-PCR assays were performed as reported by Hassan et al. (2005).

## Results

During the survey, 33 commercial orchards in five different counties were visited and 115 samples were collected and examined. Laboratory results showed that 27.82% of the samples were either singly or mixed infected by any of the tested viruses. The single infection rate (20.00%) was higher than mixed infections (7.82%). Single infection of ASPV, ACLSV and ASGV were found in 12.17%, 5.21% and 2.60% of the samples, respectively, while mixed infections of

ASPV+ASGV, ASPV+ACLSV, and ASPV+ASGV+ACLSV

were in 2.60%, 3.47% and 1.73% of the samples, respectively.ApMV was not found in any tested samples. Infected trees were marked and observed monthly during the whole vegetative period for two years. The observed symptoms were evaluated in comparison with the laboratory results. During the study; leaf mosaics, leaf deformation, fruit malformation, gummy fruit, dwarfing of the tree, bud-union abnormalities and trunk deformations were observed.

ELISA: According to the ELISA results; there was no ApMV infection, whereas ASPV is the dominant virus in quince orchards, and mixed virus infections werefound only in the Kahramanmaraş region. Consequently 32 of the 115 collected samples were found infected with one or more viruses.(Table 1).

**Tab. 1** ELISA results for quince samples collected during the survey.

| | | | Single infection | | | | Mixed infection | | | % of infection |
|---|---|---|---|---|---|---|---|---|---|---|
| **Name of province** | **Num. of orchards** | **Num. of samples** | **ApMV** | **ACLSV** | **ASPV** | **ASGV** | **ACLSV ASPV** | **ASPV ASGV** | **ACLSV ASPV ASGV** | |
| K. maraş | 17 | 67 | - | 2 | 14 | 3 | 4 | 3 | 2 | 41,79 |
| Adana | 5 | 15 | - | - | - | - | - | - | - | - |
| Niğde | 4 | 12 | - | 2 | - | - | - | - | - | 16,66 |
| Mersin | 4 | 12 | - | 1 | - | - | - | - | - | 8,33 |
| Osmaniye | 3 | 9 | - | 1 | - | - | - | - | - | 11,11 |
| Total | 33 | 115 | 0 | 6 | 14 | 3 | 4 | 3 | 2 | 32 |
| % infection | | | - | 5,21 | 12,17 | 2,60 | 3,47 | 2,60 | 1,73 | 27,82 |
| %single & mixed infection | | | | % 20 | | | | % 7,82 | | |

RT-PCR: RT-PCR tests confirmed the presence of ASPV, ASGV and ACLSV detected previously by ELISA. The image of DNA bands for ACLSV (712 bp), ASPV (414 bp) and ASGV (300) bp) is showed in Figure 1.

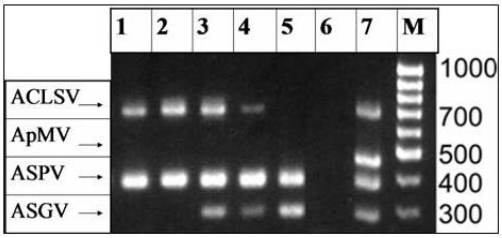


**Fig. 1** Representative results obtained by multiplex RT-PCR assay for four viruses in quince samples. Lanes 1 to 5 are quince sample infected with different virus combinations. Lanes 6 is healthy quince and line 7 is positive virus controls.

Symptomology: Visual observations of infected quince trees showed that; ASPV and ASGV infections resulted in severe leaf deformation (Fig. 2), mosaic (Fig. 3) and over-growth of the trunk (Fig. 8). Single ASPV infection cause gummy tissues on the surface of the fruit, gritty and gummy tissues around the seed, fruit malformation and trunk flexion (Fig. 4, 5, 6, 7).

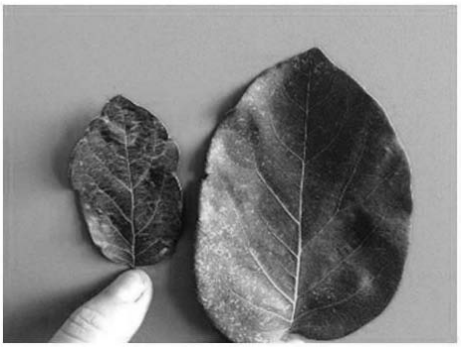

**Fig. 2** Mixed infection causes small and deformed leaves. These leaves also have some mosaic symptoms in a short period of spring.

**Fig. 3** Some infected trees gives normal size leaves but the mosaic symptoms remain for a long period till mid July.

**Fig. 4** ASPV infection causes gummy tissues on the surface of the fruit, and fruit has a rusty appearance.

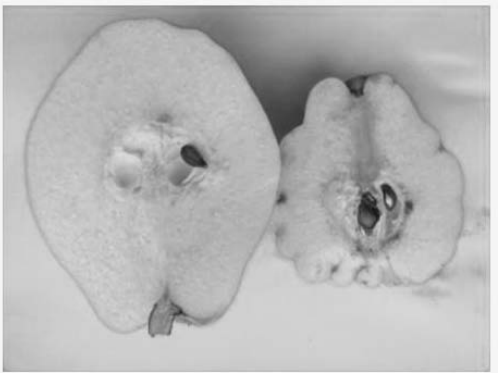

**Fig. 5** The tissue around the seed became very hard and gummy with ASPV infection.

**Fig. 6** ASPV+ASGV infection cause very severe fruit malformation. Those fruits were very hard to cut and unpleasant.

**Fig. 7** Trunk flexion observed only on ASPV infected trees.

**Fig. 8** Bud-union oversize symptoms have been observed on trees infected with both ACLSV+ASPV.

## Discussion

This study showed that ACLSV, ASPV and ASGV viruses are present in commercial quince orchards in Mediterranean region of Turkey. There was no ApMV found neither by RT-PCR nor by ELISA in collected samples from commercial quince orchards. Different types of symptoms were observed on virus infected trees and the observed symptoms were evaluated in comparison with the laboratory results. During the study; leaf mosaics, leaf deformation, fruit malformation, gummy fruit, dwarfing of the tree, gritty tissues in the fruit, bud-union abnormalities and trunk deformations were observed assumed to be the result of virus infection. Symptoms were more severe in the case of mixed infection, and more than 25% of the fruits of infected trees were not marketable. All symptoms were observed on a local quince variety that known as 'Ekmek'. This study showed that virus diseases have an important impact on quince production because, due to virus infection, fruits are becoming unmarketable and trees are becoming more weak and amorphous. Results showed that for quince production more severe damage occurs for ASPV+ACLSV mixed infection. Because of severe fruit symptoms and leaf misshapenness productive trees are losing their ability to bear fruit. It is thought that virus infections of quince are caused by infected plant propagating material. Because the production material which is used for planting quince orchards is not certified, and growers are used to using cuttings from their orchards to produce new production materials.

## Acknowledgements

Thanks to The Scientific and Technological Research Council of Turkey (TUBITAK) for funding this research activity via scientific research project. No: 1001-16O303.

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