



Mixed infections of pome and stone fruit viroids in cultivated and wild trees in Greece

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Received: 01 Jun 2012. **Published:** 15 Sep 2012. **Keywords:** apple scar skin, hop stunt, pear blister canker

In a survey during 2006-2009, 947 field samples (bark, fruit and leaves) of cultivated and wild pome and stone fruit trees showing various symptoms, including scar skin, dappling, fruit malformation, leaf mottle and stem canker (Fig. 1) were collected in different regions of Greece (Attica, Crete, Macedonia, Peloponnesus and Thessaly). Samples were tested for the presence of *Apple scar skin viroid* (ASSVd), *Hop stunt viroid* (HSVd) and *Pear blister canker viroid* (PBCVd), using tissue-print molecular hybridisation, reverse transcription-polymerase chain reaction (RT-PCR), cloning and sequencing.

Tissue-print molecular hybridisation showed that approximately 400 trees were infected with only one viroid whereas 63 trees were co-infected by two or three viroids. RT-PCR confirmed mixed infections in 26 trees but the results were not clear for samples from the other trees. Fourteen trees in all, seven pear (*Pyrus communis*), three wild pear (*Pyrus amygdaliformis*), three apple (*Malus domestica*) and one quince (*Cydonia oblonga*) were infected with ASSVd and PBCVd; five sweet cherry trees (*Prunus avium*) were infected with ASSVd and HSVd, and seven trees, four apple and three wild apple (*Malus sylvestris*) were infected with ASSVd, PBCVd and HSVd. All three viroids were sequenced in two trees, ASSVd and PBCVd in a further six trees, and ASSVd and HSVd (partially) in a wild apple tree (Table 1).

Our findings reveal for the first time natural mixed infections of apple and wild apple with ASSVd, PBCVd and HSVd, of quince with ASSVd and PBCVd and of wild apple and sweet cherry with ASSVd and HSVd. Mixed viroid infections of ASSVd, PBCVd and *Peach latent mosaic viroid* (PLMVd) in pear and wild pear (Kyriakopoulou *et al.*, 2001), of PLMVd and HSVd in peach (*Prunus persica*) (Hassan *et al.*, 2004; Tessitori *et al.*, 2002) and of PBCVd, PLMVd and HSVd in pear (Fekih-Hassen *et al.*, 2004) have been reported previously. The presence of natural viroid infections in wild species away from human influence in Greece suggests that ASSVd, PBCVd and PLMVd originated in wild hosts and are probably native to Greece (Kyriakopoulou *et al.*, 2001); our data for ASSVd, PBCVd and HSVd support this hypothesis (Kaponi 2009).

Mixed infections favour viroid recombination and evolution, and wild species used as rootstocks for cultivated species may serve as viroid reservoirs, making phytosanitary control critical (Randles, 2003).

Acknowledgements

This research project was co-financed by the European Union's European Social Fund (75%) and the Greek Ministry of Development - GSRT (25%). We also thank Dr. Francesco Faggioli, Dr. Marina Barba (CRA, Italy) and Dr. Cleanthes Israelides (NAGREF, Greece) for their support.

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Figure 1

Table 1. GenBank Accession Nos. of viroid sequences from pome and stone fruit trees in Greece.

Species and variety, district - region	ASSVd	PBCVd	HSVd
<i>Malus domestica</i> 'Royal Gala', Pella - Macedonia	GQ249349	FJ974086	GQ249348 EU925589
<i>Malus sylvestris</i> , Pella - Macedonia	FJ974100	FJ974085	FJ974075 FJ974076 EU925590
<i>Malus domestica</i> 'Golden Delicious', Pella - Macedonia	FJ974098 GQ249347	FJ974088	
<i>Malus sylvestris</i> , Pella - Macedonia	FJ974099	GQ141740	
<i>Malus sylvestris</i> , Pella - Macedonia	FJ974101		JX401927
<i>Malus domestica</i> , North Athens - Attica	FJ974102	FJ974090	
<i>Pyrus communis</i> 'Krystalli', Achaia - Peloponnese	FJ974097	FJ974103	
<i>Pyrus communis</i> 'Kontoula', Argolis - Peloponnese	FJ974091 FJ974092 FJ974093	FJ974082	
<i>Pyrus communis</i> 'Kontoula', Corinthia - Peloponnese	FJ974094	FJ974087	

Figure 2

To cite this report: Kaponi M, Luigi M, Kyriakopoulou PE, 2012. Mixed infections of pome and stone fruit viroids in cultivated and wild trees in Greece. *New Disease Reports* **26**, 8. [http://dx.doi.org/10.5197/j.2044-0588.2012.026.008]

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