Experimental Evidence that *Apple Dimple Fruit Viroid* Does not Spread Naturally

M. Malfitano, D. Alioto, A. Ragozzino
Facoltà di Agraria
Università di Napoli "Federico II"
Dip. AR.BO.PA.VE.
80055 Portici (Na)
Italy

R. Flores
Inst. Biol. Mol. Cel. de Plantas
Universidad Politécnica-CSIC
46022 Valencia,
Spain

F. Di Serio
Ist. Virologia Vegetale, CNR
Dip. Prot. Piante e Microbiol. Appl.
Università di Bari, 70126 Bari
Italy

Keywords: viroids, ADFVd, apple, pear

Abstract

A disease, named apple dimple fruit (ADF), was reported in Southern Italy first in trees of cultivar 'Starking Delicious' and later in 'Royal Gala' and 'Annurca'. The symptoms are roundish and depressed green spots, 3-4 mm in diameter, scattered on the fruit skin. In some cases, the spots coalesce and large discoloured skin areas appear predominantly around the calyx end. The disease is caused by *Apple dimple fruit viroid* (ADFVd) that has been experimentally transmitted to other commercial apple cultivars, in which it induces typical symptoms or no symptoms at all, and to the 'Fieud 37' pear indicator in which no symptoms develop. Here we report that dot- and northern-blot hybridizations of extracts from different apple and pear cultivars collected in several areas of Campania (Italy) revealed the presence of ADFVd in 'Starking Delicious' trees with typical fruit symptoms and in an 'Annurca' tree without symptoms. The distribution and spread of the viroid, monitored over a three-year period, showed a scattered pattern of infected field trees and lack of transmission between adjacent or remote trees. ADFVd was not detected in seedlings obtained from symptomatic fruits of an ADFVd-infected tree. Molecular hybridizations with specific probes failed to reveal the presence of *Apple scar skin viroid* (ASSVd), *Pear blister canker viroid* (PBCVd), *Hop stunt viroid* (HSVd) and *Peach latent mosaic viroid* (PLMVd) in the apple trees tested.

INTRODUCTION

Apple dimple fruit disease (ADF) was first reported in Southern Italy on the cultivar 'Starking Delicious' (Di Serio et al., 1996) and then on 'Royal Gala' and 'Annurca' (Di Serio et al., 1998, 2000 and 2001). The symptoms appear as roundish and depressed green spots, 3-4 mm in diameter, scattered on the red skin (Fig. 1). In some cases, the spots coalesce and large discoloured skin areas are observed predominantly around the calyx end.

The disease was initially associated to *Apple dimple fruit viroid* (ADFVd) (Di Serio et al., 1996), which later on was demonstrated to be its causal agent (Di Serio et al., 2001). ADFVd, a circular RNA of 306-307 nt with a predicted quasi-rod-like structure of minimal free energy (Di Serio et al., 1996), belongs to the family *Pospiviroidae* and, within it, to the genus *Apscaviroid,* whose type species is *Apple scar skin viroid* (ASSVd) with which ADFVd shares a sequence similarity of 63.5% (Di Serio et al., 1996 and 2001).

ADFVd has been mechanically transmitted to other commercial apple cultivars, in which it may induce typical green spots or no symptoms at all (Di Serio et al., 2001), and

to 'Fieud 37' pear (*Pyrus communis* L.), an indicator for *Pear blister canker viroid* (PBCVd) in which it replicates symptomlessly (Desvignes et al., 1999). So far, there are no data on ADFVd transmission and dissemination under natural conditions and it is unknown whether this viroid infects naturally other pome fruits.

Here we report the results of a search for the presence and spread of ADFVd in different apple and pear cultivars grown in Campania (Southern Italy), together with the results of a study on seed transmission of ADFVd.

MATERIALS AND METHODS

Sources of Material

ADFVd spread was investigated by collecting during three years (2000 to 2002), 45 and 28 fruits of different apple cultivars ('Starking Delicious', 'Annurca', 'Golden' and 'Granny Smith') from two commercial orchards where ADFVd-infected trees were first detected (Di Serio et al., 1997, 2000). Moreover, 53 samples of 'Annurca' from nearby apple orchards and 19 samples of 'Annurca', 'Renetta', 'Limoncella' and 'Starking Delicious' from other areas of Campania were also collected. To ascertain the possibility of ADFVd transmission to other pome hosts, 50 pear trees of different cultivars, growing in orchards contiguous or not to the ADFVd foci, and 3 quince trees *(Cydonia vulgaris* Pers.) were also analyzed. Additionally, the presence of ADFVd was tested in 50 seedlings grown from the seeds of symptomatic fruits from an ADFVd-infected 'Starking Delicious' tree.

RNA Extraction and Dot-blot Hybridization

Total RNA was extracted with buffer saturated-phenol from symptomatic and asymptomatic apple fruits as well as from leaves of pear trees and apple seedlings, and then partitioned by chromatography on non-ionic cellulose to obtain a fraction enriched in RNAs with a high secondary structure content (Di Serio et al., 1996, 2001). Aliquots (5 µl) were applied to nylon membranes that were hybridized overnight at 70°C (in the presence of 50% formamide) with PBCVd-, *Hop stunt viroid* (HSVd)-, *Peach latent mosaic viroid* (PLMVd)- and ASSVd-specific riboprobes labeled with digoxigenin, and revealed as recommended by the supplier (Boehringer, DIG Luminescent Detection Kit for Nucleic Acids).

Analysis by Northern-blot Hybridization

In some cases, ADFVd extracts were separated by two consecutive polyacrylamide gel electrophoreses (PAGE) under non-denaturing and denaturing conditions, and revealed by ethidium bromide staining (Di Serio et al., 1996 and 2001; Flores et al., 1985). The RNAs in the second denaturing gel were transferred to a nylon membrane that was hybridized with ADFVd- and ASSVd-specific digoxigenin-labeled riboprobes.

RESULTS AND DISCUSSION

Field inspections carried out in Campania over a three-year period have evidenced the consistent appearance of ADF symptoms (Fig. 1), just in the 8 trees from two of the orchards where the disease was first recorded (Di Serio et al., 1996, 2000). Analysis by dot-blot hybridization of the symptomatic and some asymptomatic trees from the two orchards has confirmed the presence of ADFVd in the symptomatic trees ('Starking Delicious') and revealed its occurrence in one asymptomatic tree ('Annurca') growing far from the ADFVd-infected plants (Fig. 2). Except for this 'Annurca' tree, no additional trees adjacent or remote to the original foci had resulted infected. On the basis of the scattered distribution of the infected trees and since no dispersal was observed over the three-year period, it can be concluded ADFVd does not seem to spread naturally under field conditions. Most likely, this viroid may have been inadvertently transmitted by the use of infected propagation material, particularly considering that some cultivars are

tolerant (Di Serio et al., 2001) and that in others ('Annurca') ADFVd may replicate without eliciting symptoms.

On the other hand, no hybridization signals were found by dot-blot analysis in extracts from apple and pear trees grown in orchards contiguous or distant to the ADFVd foci (Fig. 2), suggesting that ADFVd occurrence on apple trees is sporadic and that commercial pear cultivars are not naturally infected.

None of the 50 seedlings originated from the symptomatic fruits of an ADFVd-infected tree resulted positive for this viroid when analyzed by dot-blot hybridization. Therefore, ADFVd does not appear to be seed-transmitted, in line with what has been previously reported for ASSVd (Howell et al., 1998; Desvignes et al., 1999).

Finally, molecular hybridizations with several viroid-specific riboprobes showed that none of the examined apple trees was infected by HSVd, ASSVd, PBCVd, or PLMVd. In the case of ASSVd, data were obtained by northern-blot analysis to discard misinterpretations resulting from cross-hybridizations due to the sequence similarity existing between this viroid and ADFVd (Fig. 3).

Literature Cited

Desvignes, J.C., Cornaggia, D., Grasseau, N., Ambrós, S. and Flores, R. 1999. Pear blister canker viroid: studies on host range and improved bioassay with two new pear indicators, Fieud 37 and Fieud 110. Plant Dis. 83:419-422.

Di Serio, F., Alioto, D., Ragozzino, A. and Flores, R. 1996. Identification and molecular properties of a 306 nucleotide viroid associated with apple dimple fruit disease. J. Gen. Virol. 77:2833-2837.

Di Serio, F., Alioto, D., Aloj, B. and Ragozzino, A. 1997. Infossatura crateriforme della mela: una nuova malattia di probabile eziologia viroidale osservata in Italia meridionale. Inf. Fitopat. 12:14-17.

Di Serio, F., Alioto, D., Ragozzino, A., Giunchedi, L. and Flores, R. 1998. Identification of apple dimple fruit viroid in different commercial varieties of apple grown in Italy. Acta Hort. 472:595-601.

Di Serio, F., Alioto, D. and Ragozzino, A. 2000. Segnalazione in Campania di un focolaio di infezione del viroide della maculatura crateriforme della mela sulle cv. Annurca e Starking Delicious. Inf. Fitopat. 6:53-56.

Di Serio, F., Malfitano, M., Alioto, D., Ragozzino, A., Desvignes, J.C. and Flores, R. 2001. Apple dimple fruit viroid: Fulfillment of Koch's postulates and symptom characteristics. Plant Dis. 85:179-182.

Di Serio, F., Malfitano, M., Alioto, D., Ragozzino, A. and Flores, R. 2003. Apple dimple fruit viroid. p. 146-149. In: A. Hadidi, R. Flores, J.W. Randles and J.S. Semancik (eds.), Viroids. CSIRO Publishing, Collingwood, Australia.

Flores, R., Duran-Vila, N., Pallás, V. and Semancik, J.S. 1985. Detection of viroid and viroid-like RNAs from grapevines. J. Gen. Virol. 66:2095-2102.

Howell, W.E., Skrzeczkowski, L.J., Mink, G.I., Nunez, A. and Wesselz, T. 1998. Non-trasmission of apple scar skin viroid and peach latent mosaic viroid through seed. Acta Hort. 472:635-639.

Figures

Fig. 1. Depressed and discoloured spots on the red skin of apple fruits of cultivar 'Starking Delicious' induced by ADFVd.

Fig. 2. Analysis by dot-blot hybridization with an ADFVd-specific digoxigenin-labeled riboprobe of RNAs extracted from different pome sources. Lane 1, asymptomatic pear leaves. Lane 2, asymptomatic leaves of apple seedlings grown from ADFVd-infected seeds. Lane 3, healthy fruit of apple cultivar 'Starking Delicious'. Lane 4, asymptomatic fruit of ADFVd-infected apple cultivar 'Annurca'. Lane 5, symptomatic fruit of ADFVd-infected apple cultivar 'Starking Delicious'.

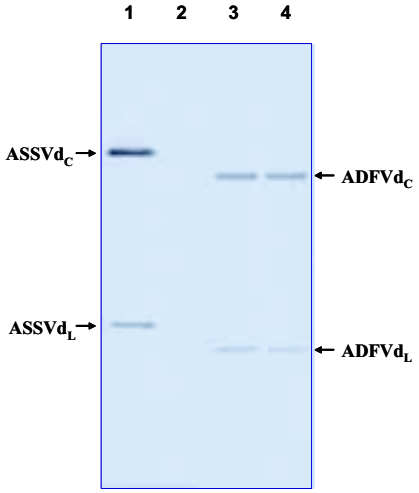

Fig. 3. Analysis by double PAGE and northern-blot hybridization with an ASSVd-specific digoxigenin-labeled riboprobe of RNAs extracted from different apple sources. Lane 1, purified ASSVd. Lane 2, healthy fruit of cultivar 'Starking Delicious'. Lane 3, fruit of cultivar 'Starking Delicious' exhibiting typical ADFVd symptoms. Lane 4, asymptomatic fruit of cultivar 'Annurca'. The position of the circular (C) and linear (L) forms of ASSVd and ADFVd RNAs are shown.