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FULL RESEARCH PAPER

An important new apricot disease in Spain is associated with *Hop stunt viroid* infection

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Abstract The province of Murcia (Murcia Region) is the one of the most important apricot growing regions in Europe. In recent years a fruit disorder named by growers as ''degeneración'' has been detected in apricot commercial orchards of this region, mainly in the variety Velázquez Fino. Affected fruits are characterized by changes in their external appearance involving rugosity and a loss of organoleptic characteristics, which leads to an unmarketable product. In order to identify the causal agent of this disorder, the presence of the most important viruses affecting stone fruit trees and *Hop stunt viroid* (HSVd) was tested. While negative results were obtained for all viruses analysed, the viroid was detected in all the symptomatic trees. Within a single tree, the viroid was restricted to branches bearing fruits with the characteristic symptoms but was usually absent from the rest of the tree. Sequence analysis of several isolates of HSVd obtained from these affected trees revealed the presence of two variants previously detected in other apricot cultivars. Taken together, these results suggest that degeneration is associated with HSVd.

Keywords *Hop stunt viroid* · Fruit quality parameters · New apricot disease · Sequence analysis · Symptomatology

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Introduction

About half the world's apricots are grown in the Mediterranean Basin where production is broadly based on a high number of local varieties. Murcia Region, located in the south east of the Spanish Mediterranean coast, is the main apricot growing area in Spain and one of the most important in Europe. As in many apricot growing areas, old orchards are found alongside new ones. Although traditional varieties are still used, recent years have seen a good deal of varietal renovation to fit new requirements of the market (Egea 1998).

The sanitary status of the apricot industry is very variable in the Mediterranean Basin but, on average, 12% of apricots tested in this area are affected by at least one virus (Myrta et al. 2006). In Spain the two most important viral diseases affecting apricot are Sharka and Viruela, which are caused by *Plum pox virus* and *Apple chlorotic*

leaf spot virus, respectively (Cambra 1999; Cañizares et al. 2001). Other viruses detected in apricot in the Murcia area belong to the *Ilarvirus* genus: *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV) with an incidence of 10%, 0.4% and 15.7%, respectively (Domínguez et al. 1998). In addition, a viroid (*Hop stunt viroid*, HSVd), which causes dapple fruit disease in plum and peach, has been reported to be present with high incidence in different apricot varieties of Murcia (Astruc et al. 1996; Cañizares et al. 1998; 2001) and in the Mediterranean Basin in general (Amari et al. 2001b; Pallás et al. 2003a). In recent years, an apricot fruit disorder known as degeneration, which causes a loss of organoleptic characteristics and reduces the commercial value, has been recorded in apricots in Murcia Region.

The first symptoms of the disorder were noted in 1985 in an orchard of Velázquez Fino that had been planted in 1975. From that moment on, the disease spread very fast and in 2004 all the trees were affected. Velázquez Fino is one of the traditional varieties of Murcia and is included in the group called clases that are characterised by the high gustatory quality of their fruits (Egea and Burgos 1996). The rapid spread of this disease and its serious economic effects led us to study its signs and evolution, in an attempt to identify the cause. Although HSVd was previously described as latent in this crop we here demonstrate that it is associated with a fruit disorder observed in apricot trees of the variety Velázquez Fino and provide evidence supporting its involvement in this pathogenic process.

Materials and methods

Plant material

The material used were trees of the Spanish apricot cv. Velázquez Fino, grafted onto seedlings of apricot cvs Real Fino, grown in two experimental orchards in Abaran (southeast Spain, 37° N, 1° W, 250 m altitude). Symptomatic fruits from affected trees located in one of the orchards were examined in order to analyze this disorder. Non-symptomatic fruits from trees located in a more recently planted orchard were also studied as negative control samples. All the trees were grown in the same conditions following the usual apricot orchard management practices of the area.

Survey for stone fruit virus

A previously described RT-PCR method (Sánchez-Navarro et al. 2005) that permits simultaneous detection and differentiation of eight important viruses that affect stone fruit trees, *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *American plum line pattern virus* (APLPV), *Plum pox virus* (PPV), *Apple chlorotic leaf spot virus* (ACLSV), *Apricot latent virus* (ApLV) and *Plum bark necrosis stem pitting associated virus* (PBNSPaV), was used to screen virus samples. The total nucleic acid extraction and RT-PCR were performed as previously described (Sánchez-Navarro et al. 2005).

Nucleic acid extraction for viroid detection

The reason behind the tissue processing procedure, which does not use organic solvent, has been described previously (Astruc et al. 1996; Pallás et al. 1987). It was adapted to handle a large number of small volume samples (Amari et al. 2001b; Cañizares et al. 1998) as follows: 0.5 g aliquots of leaf tissue were homogenized inside sealed plastic bags in the presence of 5 ml of extraction buffer (0.1 M Tris–HCl pH 8.0, 50 mM EDTA, 0.5 M NaCl, 10 mM MCE) using a hand-homogenizer. An aliquot (1 ml) of the homogenate was transferred to an Eppendorf tube, 50 µl of 20% SDS were added and the sample was incubated at 65°C for 20 min, followed by addition of 250 µl of 5 M potassium acetate and incubation on ice for another 20 min. Samples were centrifuged at 12,000 rpm for 15 min and the nucleic acids present in the supernatant were recovered by ethanol precipitation and resuspended in 40 µl of autoclaved water.

Dot-blot hybridisation

The non-radioactive digoxigenin-labelled RNA HSVd probe was obtained by T7 RNA polymerase transcription of the linearised plasmid pHSVd.EB, which contains a 272 nucleotide

residue HSVd insert. Samples were denatured at 60°C with 7.4% formaldehyde in the presence of 6× SSC, and 4 µl spots were applied to nylon membranes (Boehringer Mannheim). Prehybridization and hybridisation were carried out at 68°C as previously described (Más and Pallás 1995). In order to detect the hybridized probe, binding to anti-digoxigenin Fab fragments conjugated to alkaline phosphatase and subsequent chemiluminiscent detection using CSPD (Boehringr Mannheim) as substrate were carried out as described previously (Pallás et al. 1998).

Tissue-printing hybridisation

Tissue-printing of fruits was carried out as described previously (Amari et al. 2001a; Más and Pallás 1995). Mature fruits were freshly cut and imprinted directly onto nylon membranes, which were treated as described above.

RT-PCR amplification and sequencing of viroid isolates

RT-PCR was performed as described (Amari et al. 2001b). The primers used were the VP-19 (5′-dGCCCCGGGGCTCCTTTCTCAGGTAAG-3′, complementary to HSVd residues 60–85) and the VP-20 (5′-dCGCCCGGGGCAACTCTTCTCAGAATCC-3′, complementary to HSVd residues 78–102). Both primers lie in the strictly conserved central region of HSVd. Following RT-PCR, electrophoretic analysis confirmed the presence of a monomeric PCR product of the expected size. The PCR products were directly sequenced in both orientations in an automated DNA sequencer (ABI PRISM 337; Perkin-Elmer Corp.).

Phylogenetic analysis of HSVd sequences

Multiple alignments of nucleotide were obtained using the default options of Clustal X 1.8, a Windows interface for the Clustal W multiple sequence alignment programme. Phylogenetic analysis was made using the minimum evolution method of phylogenetic inference (Rzhetsky and Nei 1993) with 10,000 bootstrap replicates. Version 2.1 of the Molecular Evolutionary Genetics Analysis software MEGA version 2.1 was utilized (Kumar et al. 2001).

Quality index

All the fruits used were harvested at the commercial mature stage based on skin colour and fruit firmness. Immediately after harvest, fruits were transported in an air-conditioned car to the laboratory (55 km distance), where they were analysed. Three replicates of 10 fruits were analysed from both symptomatic and non-symptomatic groups. Skin and flesh colour, firmness, titratable acidity (TA), pH, soluble solids content (SSC) and weight were evaluated as quality indices. Colour values of the surface (background skin colour) and flesh, were measured with a Minolta chroma meter (CR-300, Minolta, Ramsey, NJ) tristimulus colour analyser, calibrated to a white porcelain reference plate. The colour space coordinates L^*, a^*, b^*, hue angle [H° = arctangent (b^*/a^*)] and chroma ($a^*2 + b^*2$)1/2 were determined around the equatorial region in three different positions (with an average of nine times for each apricot). The hue angle has been described as a suitable and intuitive colour index (i.e. red, yellow, blue, etc...) (Arias et al. 2000). Fruit firmness was evaluated by a compression test using a Lloyd instrument (model LR10K, Fareham Hants, UK) equipped with two (12 cm and 18 cm) flat plates. The maximum force (N) required to deform the fruit 5 mm at a speed of 25 mm min^{-1} with the slice lying on the bottom plate, was recorded. Firmness was also measured with a hand penetrometer (Kg 0.5 cm^{-2}). TA was determined by titrating 5 ml of juice with 0.1 mol L^{-1} NaOH to pH 8.1 by an automatic titration system (AOAC) (AOAC 1984). The pH values were measured using a pH meter. SSC was determined with an Atago N1 hand-held refractometer (Tokyo, Japan). Moreover, a trained panel of four experts evaluated the physical parameters such as percentage of blush on the skin, fruit surface and attractiveness as well as organoleptic parameters, such as flavour and flesh texture.

Statistical Analysis

All data are the means of three replicates made of 10 fruits, with standard deviations. Differences among fruits with symptoms and fruits without symptoms

were determined by t-Test. Statistical analysis were performed using SPSS 11.5 for Windows (Chicago, IL).

Results

Symptomatology

During recent years an apricot fruit disorder knows by the growers as ''degeneration'' has spread and gained in importance. Affected fruits are characterized by severe rugosity and decolouration of the skin (Fig. 1: apricot cv. Velázquez Fino), and the fruits do not fall so early as healthy fruit, a symptomatology that is clearly different from that caused by other pathogens. However, apart from the fruit symptomatology there is no clear symptom in the tree that characterizes this degeneration. The process of infection is gradual and very often parts of the tree show symptoms while other areas do not. From the moment that the first symptoms become evident until complete infection of the tree may take two or three vegetative cycles. Grafting assays revealed that this syndrome was transmissible. Taken together, all these observations led us to consider a potential viral origin for this fruit disorder.

Fig. 1 Apricot var. Velázquez Fino fruits from healthy and affected trees. Note that diseased fruits (left) are still green and present skin rugosity

Virus and HSVd analysis

In an attempt to identify the causal agent of this degenerative disease, leaves from symptomatic and asymptomatic apricot trees were analyzed by multiplex RT-PCR (Sánchez-Navarro et al. 2005) to test the presence of the principal viruses, which affect stone fruits trees. Negatives results were obtained for every virus analyzed: ApMV, PNRSV, PDV, APLPV, PPV, ACLSV, ApLV and PBNSPaV (data not shown).

The affected trees were tested for the presence of HSVd due to its previously reported high incidence in apricot trees in Murcia Region (Amari et al. 2001b; Cañizares et al. 1998, 2001). First, leaves from 20 symptomatic and 20 asymptomatic apricot cv. Velázquez Fino trees were analyzed by dot-blot hybridization to detect HSVd. The results showed that the viroid was present in 15 out of the 20 samples of symptomatic trees, whereas no HSVd RNA was detected in asymptomatic trees (Fig. 2). It has been

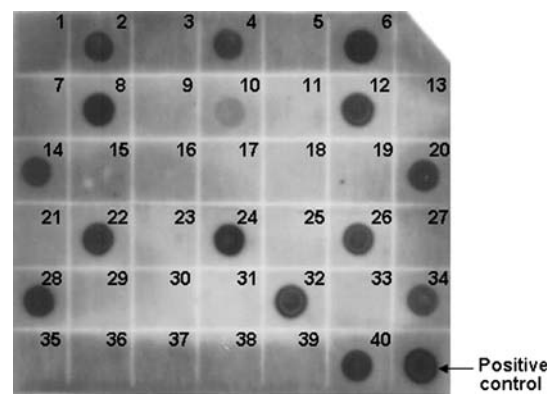

Fig. 2 Dot-blot hybridization to detect HSVd from leaves of apricot trees. Odd numbers represent leaves from different asymptomatic trees. Even numbers represent leaves from symptomatic trees. Positive control is total RNA extracted from HSVd infected cucumber

demonstrated that viruses and viroids are frequently 100 times more concentrated in the fruits of stone fruit trees than in leaves (Astruc et al. 1996; Sánchez-Navarro et al. 1998), so it is common to find no evidence of viruses or viroids in leaves, whereas levels may be quite high in fruits. We then analysed, by fruit tissue-printing hybridization, 76 fruits from different symptomatic and asymptomatic trees; HSVd was detected from all 48 fruits obtained from the symptomatic trees, while 28 of the fruits from asymptomatic trees were HSVd-free (Fig. 3: only 12 out the 48 infected fruits analyzed are shown).

When fruits from the same infected tree were analyzed, HSVd was detected in all fruits from a branch bearing symptomatic fruits; however, in the case of fruits from a branch with no visible symptomatic fruits, HSVd was only detected in an average of 2 out of 10 fruits analyzed (Fig. 4). Interestingly, the same correlation between infected fruits and the presence of HSVd was observed when the apricot varieties Mauricio and Currot were analysed (data not shown).

Characterization and phylogenetic analysis of HSVd sequence variant

HSVd was isolated from 10 symptomatic apricots and characterized. The length of all these HSVd variants was 297 nucleotides, which was in agreement with previous reports (Amari et al. 2001b; Kofalvi et al. 1997). Four out of the ten HSVd sequence variants characterized (named aprs.101, 102, 103 and 104) were found to be identical to the previously described HSVd.p2 isolated from peach and plum trees (Sano et al. 1989). The other six sequence variants characterized (apr.105 to apr.110) were found to be identical to the previously described HSVd.apr3, (isolated from Apricot cv. Bulida), which was characterized by a substitution U→C in position 59, with respect to the closest previously sequenced HSVd.p2 (Sano et al. 1989; see Fig. 2 in Amari et al. 2001b). Alignment and phylogenetic analysis of the ten HSVd sequence variants isolated showed that they could be included in the previously described phylogenetic group *Plum-type* (Amari et al. 2001b; Kofalvi et al. 1997; Sano et al. 1989) (Fig. 5).

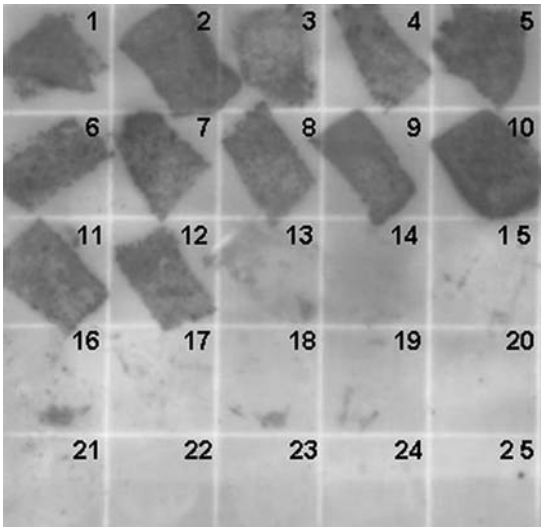

Fig. 3 Fruit tissue-printing to detect HSVd from apricot cv. Velázquez Fino. From 1 to 12, fruits from symptomatic tree. From 13 to 25, fruits from asymptomatic tree

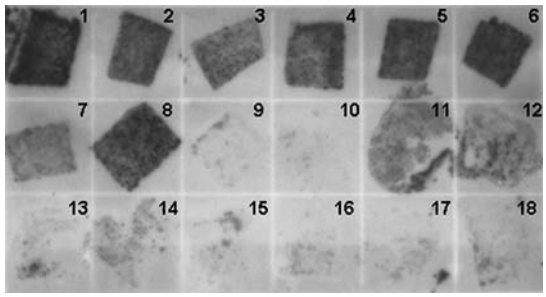

Fig. 4 Fruit tissue-printing to detect HSVd from infected apricot tree. From 1 to 8, fruits from branch with symptomatic fruits. From 9 to 16, fruits from branch with asymptomatic fruits. 17 and 18 fruits from healthy tree

Quality index

At the commercially mature stage, significant differences were found between fruits with and without symptoms in several of the quality parameters analysed (Table 1). Analysis of the background skin colour values showed that the lightness factor L^*, the b^* value and chroma (C^*) were higher in the symptomatic fruits than in the non-symptomatic ones, which indicates that colour intensity was higher in the degenerated fruits. Moreover, the percentage of blush in the skin was higher in symptomatic fruits. However, as regards flesh colour, non-symptomatic fruits had more colour than degenerated fruits, and significant differences were found between them in the case of L^*, a^* and

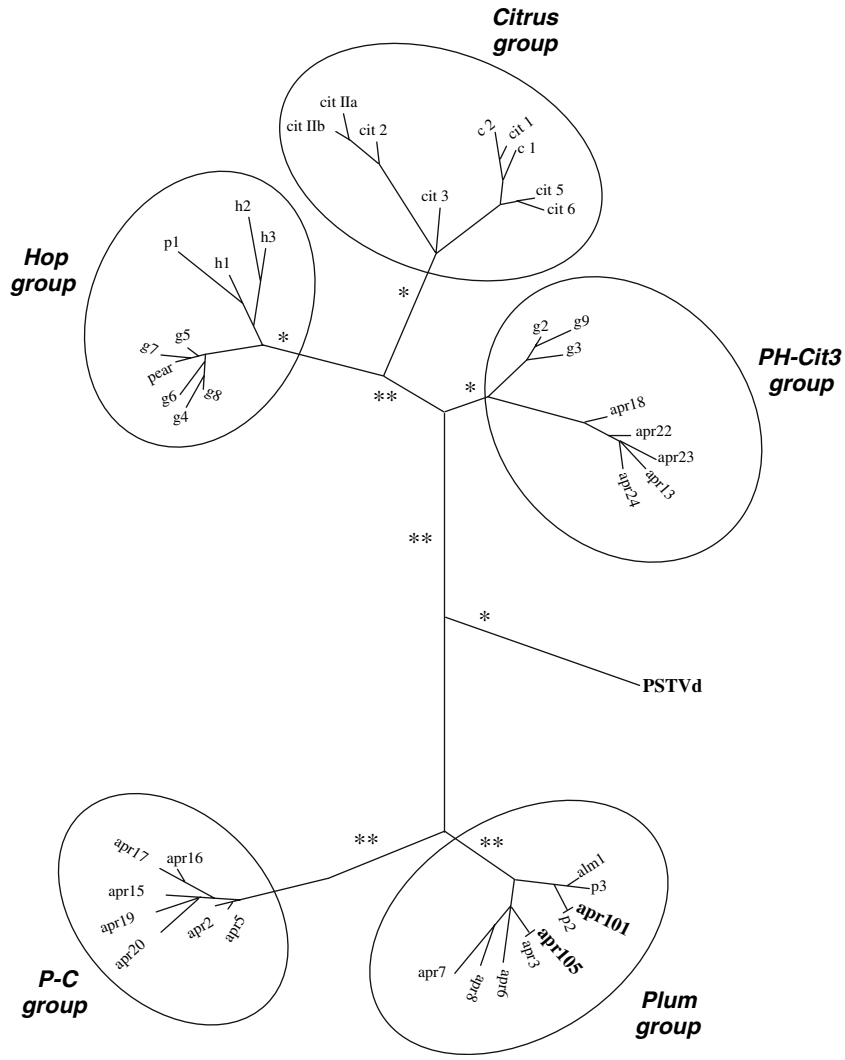

Fig. 5 Phylogenetic tree of HSVd sequence variants. Phylogenetic analysis was performed on genetic distance calculated between the two HSVd representatives variants described here (apr.101 and apr.105, in bold) and representative HSVd sequences described previously. The five phylogenetic groups described by Amari et al. 2001b, are depicted. Asterisks indicate the statistical value of the node as determined by bootstrap analysis (10,000 replicates). **: node detected in >75% of replicates; *: node detected in >50% replicates. PSTVd was included in the analysis as an out-group

hue angle (h°), which decreased from 95.78 in the symptomatic fruits to 91.56 in the non-symptomatic ones (Table 1), a yellowing that was due to carotenoid accumulation (Ruiz et al. 2005).

Fruit firmness was significantly higher in the case of the affected fruits (37.86 N measured by compression and 1.97 kg 0.5 cm^{-2} by penetrometer) whereas fruit weight was higher in the non-affected fruits (Table 1).

However, no significant differences were observed between affected and non-symptomatic fruits as regards some quality parameters. For example, titratable acidity (TA) was similar in both cases, with values of around 1.30 g malic acid/100 ml of juice. The soluble solids content (SSC) was higher in fruits without symptoms (12.80 °Brix) than in fruits with symptoms (12.50 °Brix), but the difference was not significant. With respect to the colour analysis, a^* value and h°, in the case of background skin colour, and b^* and C^* in flesh colour, these did not show significant differences (Table 1).

The trained panel of experts found significant differences concerning fruit attractiveness and especially flavour, the apricot fruit without symptoms being better appreciated (Table 1). The loss of juiciness in the degenerated fruits is one of the most important differences with regard to fruit quality, since the fruits without symptoms had a very juicy texture. Moreover, the fruit surface of Velázquez Fino without symptoms was classified as rough, while the surface in symptom-

Table 1 Quality Index Values at commercial maturity in fruits with and without symptoms from cv. Velazquez Fino[a]

	Parameters	Fruits without symptoms		Fruits with symptoms		Differences[b]	(Significance)
	Titratable acidity[c]	1.30	(0.08)	1.31	(0.16)	0.828	NS
	pH	3.89	(0.09)	3.78	(0.04)	0.018	*
	Soluble solids (%)	12.80	(0.46)	12.50	(0.85)	0.241	NS
Backgound skin colour	L*	76.62	(0.47)	81.39	(1.36)	0.000	**
	a*	−6.08	(2.48)	−7.06	(1.90)	0.312	NS
	b*	36.51	(0.19)	44.21	(0.70)	0.000	**
	h°	99.44	(3.83)	99.06	(2.37)	0.787	NS
	C*	37.07	(0.25)	44.80	(0.80)	0.000	**
	% Blush	9.45	(4.83)	17.00	(7.42)	0.049	*
Flesh colour	L*	72.21	(1.62)	80.49	(2.94)	0.000	**
	A*	−0.73	(1.32)	−2.94	(0.54)	0.000	**
	B*	29.75	(2.59)	29.54	(2.75)	0.856	NS
	h°	91.56	(2.55)	95.78	(1.56)	0.000	**
	C*	29.78	(2.58)	29.70	(2.69)	0.872	NS
	Firmness (kg 0.5 cm^{-2})	1.23	(0.38)	1.97	(0.57)	0.000	**
	Firmness (N)	28.99	(1.95)	37.86	(10.34)	0.035	*
	Weight (g)	66.34	(2.00)	52.85	(3.58)	0.005	**
	Attractiveness (1–10)	6.67	(0.58)	5.83	(0.29)	0.028	*
	Flavour (1–10)	9.00	(0.50)	6.00	(0.40)	0.007	**
	Surface	rough		warty			**
	Flesh texture	juicy		floury			**

[a] Standard deviation ($n = 3$) in parentheses

[b] Differences between fruits with and without symptoms with the *T*-test for independent samples

[c] Grams of. malic acid/100 ml juice

* differences with a probability of 95%

** differences with a probability of 99%

NS: No significant differences

atic fruits was warty, according to the panel of experts. Finally, field observations showed that symptomatic fruits at the mature stage remained on the tree longer than those without symptoms.

Discussion

HSVd has been described as an infectious agent in several plant families. In some hosts, HSVd infection is associated with serious disorders of economic importance such as hop stunt (Shikata 1990), dapple fruit disease of plum and peach (Sano et al. 1989) and citrus cachexia (Diener et al. 1988; Reanwarakom and Semanick 1999; Semancik et al. 1988). However in apricot, where a high incidence of HSVd has been reported in southeastern Spain (Cañizares et al. 1998, 2001) and the Mediterranean region in general (Amari et al. 2001b), the infection was always described as latent (Pallás et al. 2003b). Here, we report for first time, the detection of HSVd in symptomatic apricot trees cvs Velázquez Fino, Mauricio and Currot in commercial apricot orchards of Murcia, where it is associated with a disorder severely affecting apricot fruit quality especially in cv. Velázquez Fino.

The clear symptomatology observed in apricot fruits of the variety Velázquez Fino led us to carry out this research. Basically, this symptomatology consisted of a loss of fruit quality, reflected by the results of the physic-chemical and organoleptic analyses, since there were significant differences in

some of the quality parameters evaluated in the symptomatic fruits compared with fruits from healthy trees (Table 1). The symptomatic fruits weighed less and the flesh was lighter in colour. Moreover the skin showed a wartiness that made the fruit less attractive for consumers. Nevertheless, the most important quality involved loss of taste and juiciness. Many authors indicate that appearance, taste and texture are the main factors in apricot quality (Gurrieri et al. 2001; Parolari et al. 1992; Souty et al. 1990). Thus, the degradation of these three quality parameters as a result of the disease may cause severe problems in the production and marketing of apricot as well as other stone fruits.

Because the analyzed apricot trees were free of the most common stone fruit viruses we attempted to determine whether HSVd, a viroid widely reported in apricot, could be associated with the symptomatology. The hybridization assays demonstrated that HSVd was clearly present in all the symptomatic samples but not in the asymptomatic controls, there being a very close correlation between HSVd detection and apricot symptomatology. This correlation was even maintained within a single tree, when branches bearing affected fruits were compared with those in which no symptoms were apparent. Viroids in general are easily transmitted mechanically and it has been suggested that contaminated pruning tools may play a role in the spread of viroids in commercial orchards (Hadidi et al. 1997). When growers were recommended to use decontaminated tools, a significant decrease in the incidence of this degenerative syndrome was observed. All these results strongly suggest that the degeneration is caused by HSVd and experiments are in progress to demonstrate Koch's postulates.

Characterization of the HSVd isolates obtained from these infected trees revealed the presence of two different HSVd variant sequences, HSVd.apr3 and HSVd.p2, equally distributed in all the analyzed symptomatic samples, indicating that this apricot disorder cannot be correlated with a specific HSVd variant sequence. These HSVd variants clustered in the phylogenetic Plum group, whose members mainly infect stone fruits (Amari et al. 2001b; Kofalvi et al. 1997; Sano et al. 1989).

Taking into account that the HSVd variant sequences (apr.3 and p2) detected in the symptomatic apricot Velázquez Fino were also commonly found in several symptomless apricot cultivars (Bulida, Mauricio, Valenciano, Pepito and Real Fino) from Murcia Region (Cañizares et al. 1998, 2001), suggests that this apricot fruit disorder is related to the high susceptibility of this variety to HSVd infection. However, our trained panel of experts also found significant differences in fruit flavour between infected and healthy fruits from cvs Mauricio and Currot, although infected fruits did not show the same skin aspect as infected Velázquez fruits. The possibility that other factors, not considered in this work, such as viroid titre or favourable environmental conditions, could be involved in the expression of this apricot disorder cannot be excluded.

The situation observed for HSVd in apricot resembles very much the one previously described for HSVd in plum and peach (Sano 2003). HSVd has been associated with dapple fruit disease of plum and peach in Japan. It was first identified in cv. Taiyo and later became prevalent in cvs Beauty and Santa Rosa. Our work emphasizes the need to control the presence of this viroid in Spain, especially in the most widely cultivated apricot varieties, Bulida and Real Fino, due to the high incidence observed.

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References

Amari, K., Cañizares, M. C., Myrta, A., Sabanadzovic, S., Di Terlizzi, B., & Pallás, V. (2001a). Tracking *Hop stunt viroid* infection in apricot trees during a whole year by non-isotopic tissue printing hybridisation. *Acta Horticulturae, 550*, 315–319.

Amari, K., Gomez, G., Myrta, A., Di Terlizzi, B., & Pallás, V. (2001b). The molecular characterization of 16 new sequence variants of *Hop stunt viroid* reveals the existence of invariable regions and a conserved hammerhead-like structure on the viroid molecule. *Journal of General Virology, 82*, 953–962.

AOAC (1984). *Official methods of analysis*, 14th edn (pp. 414–420). Arlington, VA: Association of Official Analytical Chemists.

Arias, R., Lee, T. C., Logendra, L., & James, H. (2000). Correlation of lycopene measured by HPLC with the *L**, *a**, *b** colour readings of hydroponic tomato and the relationship of maturity with colour and lycopene content. *Journal of Agricultural Food Chemistry, 48*, 1697–1702.

Astruc, N., Marcos, J. F., Macquaire, G., Candresse, T., & Pallás, V. (1996). Studies on the diagnosis of *hop stunt viroid* in fruit trees: Identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. *European Journal of Plant Pathology, 102*, 837–846.

Cambra, M. (1999). Strategies for the control of *Plum pox virus* in apricot in the Mediterranean areas. *Acta Horticulturae, 488*, 725–730.

Cañizares, M. C., Aparicio, F., Amari, K., & Pallás, V. (2001). Studies on the aetiology of apricot viruela disease. *Acta Horticulturae, 550*, 249–255.

Cañizares, M. C., Marcos, J. F., & Pallás, V. (1998). Studies on the incidence of *hop stunt viroid* in apricot trees (*Prunus armeniaca*) by using an easy and short extraction method to analyze a large number of samples. *Acta Horticulturae, 472*, 581–585.

Diener, T. O., Smith, D., Hammond, R., Albanese, G., La Rosa, R., & Davino, M. (1988). Citrus B viroid identified as a strain of *Hop stunt viroid*. *Plant Disease, 72*, 691–693.

Domínguez, S., Aparicio, F., Sánchez-Navarro, J. A., Cano, A., & García-Bruton, J. (1998). Studies of the incidence oh *Ilarvirus* and *Apple chlorotic leaf spot virus* (ACLSV) in apricot trees in the Murcia region. *Acta Horticulturae, 472*, 203–210.

Egea, J. (1998). El albaricoquero en España: Panorámica varietal. *Fruticultura Profesional, 96*, 49–55.

Egea, J., & Burgos, L. (1996). Detecting cross-incompatibility of three North American apricot cultivars and establishing the first incompatibility group in apricot. *Journal of the American Society for Horticultural Science, 121*(6), 1002–1005.

Gurrieri, F., Audergon, J. M., Albagnac, G., & Reich, M. (2001). Soluble sugars and carboxylic acids in ripe apricot fruit as parameters for distinguishing different cultivars. *Euphytica, 117*, 183–189.

Hadidi, A., Giunchedi, L., Shamloul, A. M., Poggi-Pollini, C., & Amer, A. M. (1997). Occurrence of *Peach latent mosaic viroid* in stone fruits and its transmission with contaminated blades. *Plant Disease, 81*, 154–158.

Kofalvi, S. A., Marcos, J. F., Cañizares, M. C., Pallás, V., & Candresse, T. (1997). *Hop stunt viroid* (HSVd) sequence variants from Prunus species: Evidence for recombination between HSVd isolates. *Journal of General Virology, 78*, 3177–3186.

Kumar, S., Tamura, K., Jacobsen, I. B., & Nei, M. (2001). MEGA2: Molecular evolutionary genetics analysis software. *Bioinformatics, 17*, 1244–1245.

Más, P., & Pallás, V. (1995). Non-isotopic tissue-printing hybridization: A new technique to study long-distance plant virus movement. *Journal of Virological Methods, 52*, 317–326.

Myrta, A., Di Terlizzi, B., Savino, V., & Pallás, V. (2006). Viruses and Viroids of apricot in the Mediterranean: Incidence and biodiversity. *Acta Horticulturae, 107*, 409–417.

Pallás, V., Amari, K., Gomez, G., Ghanem-Sabanadzovic, N., & Di Serio, F. (2003a). Viroids of stone fruits: Incidence and diseases in the Mediterranean region. *Options Méditerranéennes, Serie B,45*, 129–133.

Pallás, V., Gómez, G., Amari, K., Cañizares, M., & Candresse, T. (2003b). Hop stunt viroid (HSVd) in apricot and almond. In A. Hadidi, J. Randles, J. Semancik, & R. Flores (Eds.), *Viroids* (pp. 168–170). St Paul, USA: Science Publisher.

Pallás, V., Más, P., & Sánchez-Navarro, J. A. (1998). Detection of plant RNA viruses by non-isotopic dot-blot hybridization. In G. Foster & S. Taylor (Eds.), *Plant virus protocols: From virus isolation to transgenic resistance* (pp. 461–468). Totowa, NY, USA: Humana Press.

Pallás, V., Navarro, A. J., & Flores, R. (1987). Isolation of a viroid-like RNA from hop different from hop stunt viroid. *Journal of General Virology, 68*, 3201–3205.

Parolari, G., Virgili, R., & Bolzoni, L. (1992). Analysis of sensory and instrumental data on apricot purees with pattern recognition techniques. *Analytical Chemistry Acta, 259*, 257–265.

Reanwarakom, K., & Semancik, J. S. (1999). Correlation of HSVd variants to cachexia and xilosporosis disease of citrus. *Phytopathology, 89*, 568–574.

Ruiz, D., Egea, J., Tomas-Barberan, F. A., & Gil, M. I. (2005). Carotenoids from new apricot (*Prunus armeniaca* L.) varieties and their relationship with flesh and skin colour. *Journal of Agricultural and Food Chemistry, 53*, 6368–6374.

Rzhetsky, A., & Nei, M. (1993). Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Molecular Biological Evolution, 10*, 1073–1095.

Sánchez-Navarro, J. A., Aparicio, F., Herranz, M. C., Minafra, A., Myrta, A., & Pallás, V. (2005). Simultaneous detection and identification of eight stone fruit viruses by one-step RT-PCR. *European Journal of Plant Pathology, 111*, 77–84.

Sánchez-Navarro, J. A., Aparicio, F., Rowhani, A., & Pallás, V. (1998). Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of *Prunus necrotic ringspot virus* in herbaceous and Prunus hosts. *Plant Pathology, 47*, 780–786.

Sano, T. (2003). Hop stunt viroid in plum, peach. In A. Hadidi, J. Randles, J. Semancik, & R. Flores (Eds.), *Viroids* (pp. 168–170). St Paul, USA: Science Publisher.

Sano, T., Hataya, T., Terai, Y., & Shikata, E. (1989). *Hop stunt viroid* strains from dapple fruit diseases of plum and peach in Japan. *Journal of General Virology, 70*, 1311–1319.

Semancik, J. S., Roistacher, C. N., Rivera-Bustamante, R., & Duran-Vila, N. (1988). Citrus cachexia viroid, a new viroid of citrus: Relationship to viroids of the exocortis disease complex. *Journal of General Virology, 69*, 3059–3068.

Shikata, E. (1990). New viroids fron Japan. *Seminars of Virology, I*, 107–115.

Souty, M., Audergon, J. M., & Chambroy, Y. (1990). Abricot: Les critères de qualité. *L'Arboriculture fruitière, 430*, 18–24.