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Effect of a traditional control method (tree removal) on the spread of sharka in an apricot orchard in Southeastern Spain

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Summary. The spatial spread of sharka disease (*Plum pox virus*, PPV) was studied from 1990 to 2002 in a traditional apricot (*Prunus armeniaca* L.) orchard located in Murcia (Southeast of Spain). The occurrence of sharka was determined by the visual observation of symptoms on leaves and fruits during the ripening stage, when symptoms are most visible. To ascertain PPV in doubtful samples, an ELISA-DASI assay was applied to the leaves. All trees infected the previous year were pulled up and were not replanted. Results showed that the virus was introduced to the farm by natural vectors (aphids) from a neighbouring sharka-infected plum orchard. It was then transmitted from these infected trees mainly to nearby trees by the same vectors, although also often to trees standing quite a distance away. The long interval between infection and symptom appearance makes eradication of the disease more difficult. Pulling up infected trees as a control method reduced the percentage of trees ultimately lost, and over the long term could stop the further spread of the disease.

Key words: *Prunus armeniaca*, *Plum pox virus*, epidemiology, control.

Introduction

Sharka, caused by *Plum pox virus* (PPV), is the most serious viral disease affecting apricot, plum and peach trees in Europe (Nemeth, 1994; Roy and Smith, 1994). The damage produced depends on the species, the cultivar and the viral strain, and ranges from chlorosis of the leaves to total deformation of the fruit, which becomes unmarketable (Pelet and Bovey, 1968). Described for the first time in Bulgaria in 1917, it spread throughout Europe, North Africa, India and Chile (Németh, 1994), and more recently to North America (Levy *et al.*, 2000).

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PPV is characterized by wide genetic variability, but in Europe there are two major strains, Dideron (PPV-D) and Marcus (PPV-M) (Candresse *et al.*, 1994). Only the PPV-D strain has been isolated in Spain (Kölber, 2001), South America (Chile) (Reyes *et al.*, 2001) and North America (USA and Canada) (Damsteegt *et al.*, 2001). Other less common PPV isolates include El Amar (PPV-E) found in North Africa, and Cherry (PPV-C) in central Europe (Kölber, 2001).

Spain is the European Union's biggest producer and exporter of apricots, and the Region of Murcia, in the southeast of the country, produces more than 60% of the national total, with 13,000 ha under cultivation and a production of around 120,000 tonnes per year (MAPA, 1998). In this region, apricots are grown in small orchards, often with more than one cultivar per orchard. The PPV-D strain

of sharka was first detected in Spain in 1984 in Seville, Valencia, Murcia and Lérida (Llácer *et al.*, 1985) and is at present the most serious disease of apricots in the country. In the regions of Murcia and Valencia, it causes important economic losses in apricot and plum trees, estimated at around 6 million euro only for assistance with pulling up affected trees from 1991 to 1997 (Dicenta *et al.*, 1998).

Given the way in which sharka is transmitted, naturally by aphids, or through the use of infected plant material by man (Labonne *et al.*, 1988; Audergon *et al.*, 1989; Keck *et al.*, 1992; Avinet *et al.*, 1994), control is very difficult. Until good new resistant cultivars are developed, all we can do is to reduce the rate of sharka spread as much as possible.

Epidemiological studies on the natural spread of PPV have been performed in non-isolated highly infected orchards for short periods of time (2–4 years) in different European countries, including Spain (Llácer *et al.*, 1992; Gottwald *et al.*, 1995; Dicenta *et al.*, 1999a), France (Labonne and Quiot, 1987; Audergon *et al.*, 1989), Greece (Varveri *et al.*, 1999), and the Czech Republic (Blazek *et al.*, 2002). However, no long-term studies (5 years or more) have so far been carried out.

The traditional method to control sharka starts with the identification of infected trees by visual observation of symptoms on the leaves and fruits. Farmers must notify the local authority of the appearance of any diseased trees. Identification is confirmed with the ELISA detection technique, and confirmed infected trees are then cut down and removed (Dicenta *et al.*, 1999b).

In this study, the spatial spread of sharka was monitored for a long period (13 years) in an almost completely isolated orchard in SE Spain, with only one source of infection and where the traditional method of control was being followed. The aim was to ascertain whether the traditional control procedure was an effective means for controlling the spread of sharka disease in infected fields.

Materials and methods

Experimental orchard

An almost completely isolated apricot orchard infected with the PPV-D strain, the only strain so far found in Murcia (Dicenta *et al.*, 1999b), was studied. The orchard was located in Murcia (Southeast of Spain) and had an area of about 1 ha on which grew some 150 trees of three traditional apricot cultivars grafted on Real Fino rootstock. Fruits were thinned and harvested by hand, and marketed fresh. In 1990, when this study was started, there were 40 20-year-old Guillermo trees, 34 20-year-old Velázquez de Upa trees, and 76 10-year-old Velázquez trees. Apricot trees were spaced 8×8 m. The orchard was bordered by mountains on three sides, and on the fourth side by an olive orchard flanked by a Santa Rosa plum orchard on its right (Fig. 1).

Evaluation and control of sharka

The occurrence of sharka in each tree was determined by visual observation of symptoms on the leaves in early spring, and on the fruits during the ripening stage in May and early June. At this time symptoms are most visible in both leaves and fruits. The fact that fruit was thinned and harvested by hand indicated a rigorous subsequent monitoring of the appearance of PPV symptoms in the tree.

To ascertain the occurrence of *Plum pox virus* in the doubtful samples an ELISA-DASI (Double Antibody Sandwich Indirect) assay was applied to the leaves using 5B monoclonal antibody against the coat protein of PPV in accordance with the protocol of Cambra *et al.* (1994). Micro-plates (Nunc, Barcelona, Spain) were incubated at 37 °C for 2 h with polyclonal rabbit antibodies (1.42 mg ml^{-1} in 1%, w:v) (Real-Durviz, Valencia, Spain) and carbonate buffer (0.159% Na_2CO_3, 0.293% $NaHCO_3$, pH 9.6). Virus extractions were carried out using 1 g of leaf in 5 ml of extraction buffer (2 g Dieca and 20 g PVP-10 in 1000 ml PBS [0.08% NaCl, 0.002% KH_2PO_4, 0.3% $Na_2HPO_4 \cdot 12H_2O$, 0.02% KCl, pH 7.4]). Samples were incubated at 5 °C for 16 h. After washing for 3×5 min with PBS-Tween-20 (0.5 ml l^{-1} Tween-20), micro-plates were incubated at 37 °C for 2 h in 1% (w:v) bovine serum albumin (BSA) (Roche, Barcelona, Spain)-PBS with the specific monoclonal antibodies (0.1 mg ml^{-1}). After washing 3 times with PBS-Tween-20, samples were incubated in 1% (w:v) BSA-PBS with an alkaline phosphatase-labelled antibody (0.1 mg ml^{-1}) at 37 °C for 2 h. Then the micro-plates were washed again and developed with a p-nitrophenolphosphate colorimetric substrate (Sigma, Madrid, Spain), recording the optical densities (OD) at 405 nm after

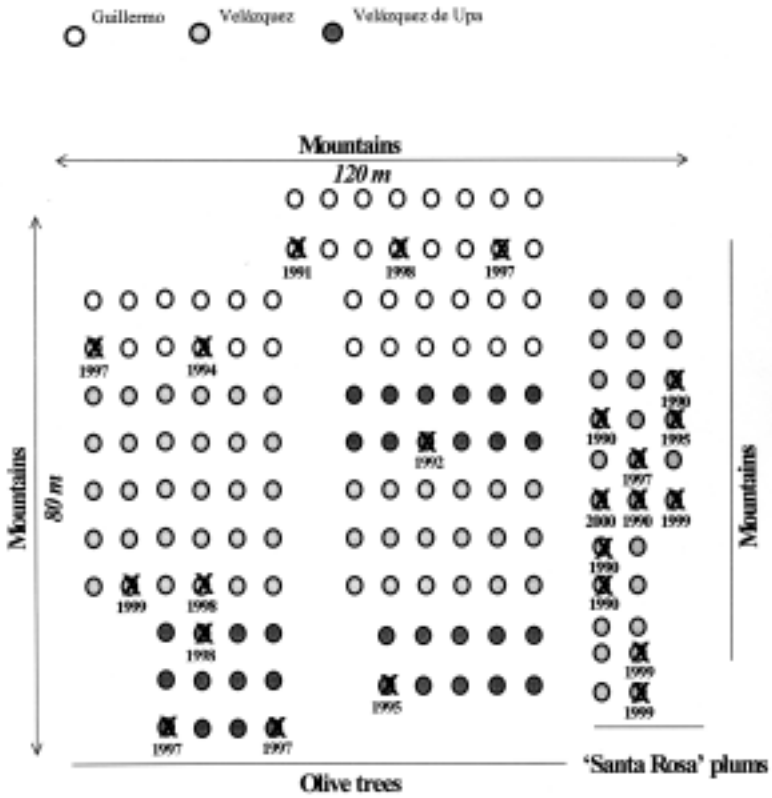

Fig. 1. Spatial and temporal distribution of diseased trees in the orchard studied. The year of removal is shown below each symptomatic and therefore removed tree (×).

60 min. In accordance with Sutula *et al*. (1986), samples with an OD double that of the healthy control were considered ELISA-positive.

All trees infected in the previous year were pulled up as part of the control method, but they were not replanted. The study lasted 13 years (1990–2002). A map was made showing the location of each tree in the orchard. On this map each year's situation was recorded, and the year of removal of any diseased trees was marked.

Results and discussion

As regards spatial spread of sharka, trees that became infected were initially (1990) located mainly on the right side of the orchard near the Santa Rosa plum orchard. In general, new sharka infections appeared in trees in the area fairly close to the plum orchard, but some infections also occurred in trees further away. For example, the infected tree removed in 1991 was located 100 m from those removed in 1990. It was noticeable that the disease had spread to a large part of the orchard after 4 years of study (Fig. 1).

As regards the time course of sharka spread, the greatest number of infected trees was detected in 1990 at beginning of the study. Five trees showed symptoms in that year. In the immediately following years, the number of removed trees was much smaller, one in 1991 and one in 1992. No new infections were detected in 1993 or 1996, while in 1994 there was one, and in 1995 two. However, a high increase of removed trees was then observed in 1997 (5), 1998 (3), and 1999 (4). After that, the infection again went down, to only one tree removed in 2000, and none in 2001 and 2002 (Fig. 1 and 2).

Cumulative infection levels (from 1990 to 2002) were similar between cultivars: 12.5% in Guillermo, 14.6% in Velázquez de Upa and 17.1% in Velázquez. The slightly higher infection percentage in Velázquez could be due to its greater proximity to the initial infectious focus, the Santa Rosa plum orchard. These levels confirmed the high susceptibility of all three Spanish cultivars evaluated

(Martínez-Gómez and Dicenta, 1999).

Figure 3 shows the cumulative percentage of sharka-infected trees. In the orchard under study, where the disease was controlled by tree removal, this percentage rose slowly from an initial 3.3% in 1990 to 15.3% 13 years later. These percentages are very low in comparison with final cumulative percentages of infection in other non-isolated orchards in severely affected areas in Spain, such as 100% reported by Llácer *et al*. (1992), and 40% by Dicenta *et al*. (1999a), or in Greece, with 56% found by Varveri *et al*. (1999). The annual rate of infection, an average of 1% during the 13-years study, was less than that reported by Blazek *et al*. (2002) for isolated plum orchards in the Czech Republic, where it was between 3 and 10%. On the other hand, Varveri *et al*. (1999) found that when orchards were isolated, disease progress was slower.

The disease seemed to be spread to the orchard by aphids from the neighbouring Santa Rosa plum orchard. This can be assumed because the first infected trees in the orchard grew near the plum orchard. Leaves and fruit with slight sharka symptoms were also observed in the plum trees of this orchard in 1990 and 1991. These symptoms were also observed in other years during the study. However the symptoms in the plum leaves and fruit were not monitored overtime. In this cultivar, as in other plum cultivars, the fruit of sharka-infected trees does not present distinctly noticeable symptoms (Llácer, 1987); for this reason farmers often continue to cultivate infected plum trees. This practice, which appears beneficial in the short term (since trees are not removed and continue to produce fruit), is however detrimental in the long run.

The appearance of newly infected trees in 1999, again in the area near the plum orchard, seemed to indicate that the plum orchard was a recurrent inoculum source. From there, the disease was spread further by aphids to the rest of the apricot orchard. That sharka is transmitted by aphids is well known, PPV being the only fruit-tree virus spread in this way, which makes its elimination more difficult (Labonne and Quiot, 1987; Labonne *et al*., 1988; Audergon *et al*., 1989). The most abundant aphid species in the apricot orchard in spring and early summer were *Myzus persicae* and *Hyalopterus pruni*. Less common species were *Aphis gossyppii*, *A. faba* and *A. spiraecola*. All these aphid species with the exception of *H. pruni* have been reported to be efficient PPV vectors (Avinent *et al*., 1994).

Aphids spread the infection not only to trees bordering the previous focus of infection, but also to trees further away. This finding partially agreed with Dicenta *et al*. (1999a), who reported that PPV was transmitted from diseased trees to neighbouring trees by vectors. Blazek *et al*. (2002) found a relation between the distance of the trees from a source of infection and the rate of infection: infection was greater when the source was less than 5 m away. By contrast, Gottwald *et al*. (1995) found that sharka tended to infect trees that were further away more than trees that were closer.

Diseased trees did not usually show signs of sharka until 1 or 2 years after becoming infected, as was found in this study and is mentioned by other authors (Marenaud and Yürektürk, 1974; Morvan, 1988). This lag in the symptom appearance behind infection, combined with the extremely irregular distribution of the virus in its ligneous host (Albrechtova, 1986; Audergon *et al*., 1989; Martínez-Gómez and Dicenta, 2001), which was also observed in our study, makes eradication of the virus extremely difficult since infected trees do not show symptoms in the first years and are therefore not thought to be diseased, and are not pulled up. Moreover, the foliar symptoms of sharka disappear when temperatures rise (Breniaux *et al*., 1990) which makes visual detection even more difficult.

Conclusions

The systematic removal of diseased trees was an effective way to control sharka. In orchards where this measure is not strictly applied the number of infected trees increases year after year, with worse consequences for the farmer and his neighbours eventually (Llácer *et al*., 1992; Dicenta *et al*., 1999b; Varveri *et al*., 1999; Blazek *et al*., 2002). Farmers are often reluctant to apply removal because it means losing trees that are still bearing. In this study the cumulative disease incidence (which is the cumulative proportion of trees removed from 1990 to 2002) rose from 3.3% in 1990 to about 15% in 2002. In other cases this percentage may reach 100%. In our study, however, due to the lag of symptom appearance behind infection, the disease, though not completely eradicated, was reduced to negligible levels. Apparently, if action is taken quick-

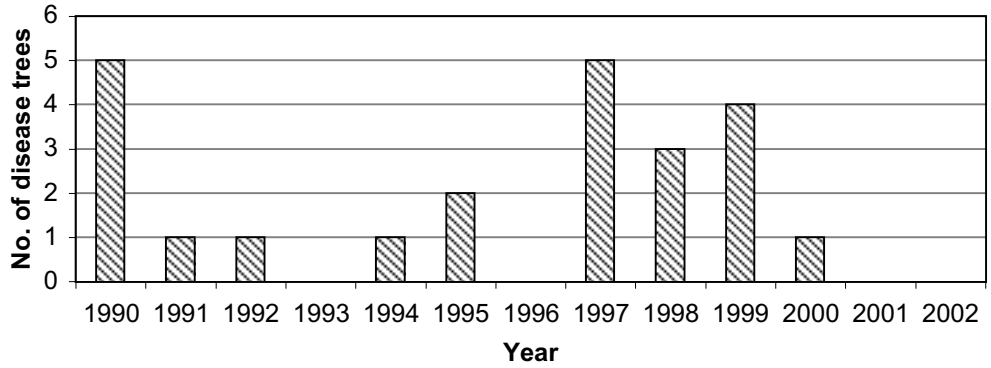

Fig. 2. Number of sharka-infected apricot trees in the orchard studied from 1990 to 2002.

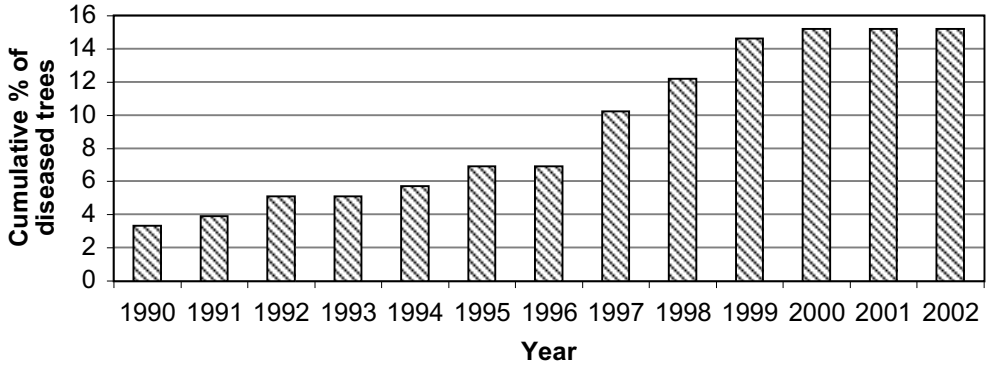

Fig. 3. Cumulative percentages of sharka-infected apricot trees in the orchard studied from 1990 to 2002.

ly, immediately upon the appearance of the first foci, when the disease is not yet widespread, it can be eradicated. Pulling up infected trees reduced the percentage of trees lost overall, and in the long term halted the spread of the disease.

Until new resistant varieties adapted to particular areas of cultivation are developed, sharka control should continue to be by detecting and pulling up infected trees. The present long-term study showed that when infected trees were systematically removed in an almost completely isolated orchard with only one source of infection (even when the original infectious focus persisted), losses were low and did not have a drastic impact on profits. This control measure should be undertaken by all orchard owners without exception and should not be left to each orchard owner individually, since sharka will spread from one orchard to another by aphids. A lack of co-operation among growers could lead to a very high rate of PPV dispersion and severe rates of infection.

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Literature cited

Albrechtova L., 1986. Investigations on the distribution of sharka virus (Plum pox virus) in tissue of *Prunus domestica*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 93, 190–201.

Audergon J.M., J. Chadoeuf, G. Labonne, P. Monestiez, G.

Morvan and J.B. Quiot, 1989. Virus de la Sharka, Eléments d'épidémiologie. Répartition spatiale du virus dans un arbre et analyse du développement en verger. *4th International Plant Virus Epidemiology Workshop*, September 1989, Montpellier, France, 200–202.

Avinent L., A. Hermoso de Mendoza and G. Llácer, 1994. Transmission of plum pox potyvirus in Spain. *EPPO Bulletin,* 24, 669–675.

Blazek J., F. Parstein and R. Karesova, 2002. Spread of Plum pox virus in new plum orchards of the Czech Republic and resistant cultivars to the disease. *XXVI International Horticultural Congress*, 11–17 August 2002, Toronto, Canada, 336 (abstract).

Breniaux D., A. Micoud and M. Ollier, 1990. *Étude de l'Éxteriorization du Virus de la Sharka sur Pêcher*. Ministère de l'Agriculture et de la Forêt, SRPV Rhône-Alpes, France.

Cambra M., M. Asensio, M.T. Gorris, E. Camarasa, J.A. Garcia, J.J. Moya, D. López-Abella, C. Vela and A. Sanz, 1994. Detection of *Plum pox potyvirus* using monoclonal antibodies to structural and non-structural proteins. *EPPO Bulletin* 24, 569–578.

Candresse T., G. MacQuaire, M. Lanneau, M. Bousalem, T. Wetzel, L. Quiot-Douine, J.B. Quiot and J. Dunez, 1994. Detection of *Plum pox potyvirus* and analysis of its molecular variability using immunocapture-PCR. *EPPO Bulletin* 24, 585–595.

Damsteegt V.S., A.L. Stone, D.G. Luster, F.E. Gildow, L.E. Levy and R. Welliver, 2001. Preliminary characterization of a North American isolate of *PPV* from naturally infected peach and plum orchards in Pennsylvania. *Acta Horticulturae* 550, 145–151.

Dicenta F., P. Martínez-Gómez, E. Abad, S. García-Vidal and G. Llácer, 1998. Estado actual de la sharka (Plum pox potyvirus) en España. *IX Congreso de la Sociedad Española de Fitopatología*. Salamanca, Spain, 245 (abstract).

Dicenta F., P.J. Pérez-Campoy, P. Martínez-Gómez, J. García-Brunton and M.A. Botella, 1999a. Natural spread of sharka disease in orchards in Murcia (Spain). *Acta Horticulturae* 488, 775–778.

Dicenta F., P.J. Pérez-Campoy, P. Martínez-Gómez, J. García-Brunton and E. Abad, 1999b. Plum pox virus situation in Murcia (Spain). *Acta Horticulturae* 488, 769–774.

Gottwald T.R., L. Avinent, G. Llácer, A. Hermoso de Mendoza and M. Cambra, 1995. Analysis of the spatial spread of sharka (plum pox virus) in apricot and peach orchards in Eastern Spain. *Plant Disease* 79, 266–278.

Keck M., A. Stöger and K. Russ, 1992. Investigations on the spread of sharka in view of a systematic virus control. *Acta Horticulturae* 309, 135–137.

Kölber M., 2001. Workshop on Plum pox. *Acta Horticulturae* 550, 249–255.

Labonne G. and J.B. Quiot, 1987. L'épidémiologie des virus transmis sur le mode non-persistant. *Annales ANPP* 2, 245–272.

Labonne G., J.B. Quiot and I. Hubert, 1988. Les pucerons vecteurs de la sharka dans un verger d'abricotiers du sud-est de la France. Agriculture. L'abricotier. Ed. UE. 25–32 pp.

Levy L., V.S. Damsteegt and R. Welliver, 2000. First report of *Plum pox virus* (sharka disease) in *Prunus persica* in the United States. *Plant Disease* 84, 202.

Llácer G., 1987. La sharka una grave amenaza para la producción de albaricoques en España. *Fruticultura Profesional* 12, 41–47.

Llácer G., M. Cambra and A. Laviña, 1985. Detección y primeros estudios del virus de la sharka en España. *Anales Instituto Nacional de Investigaciones Agrarias* 28, 157–166.

Llácer G., L. Avinent and A. Hermoso de Mendoza, 1992. Epidemiology of plum pox (sharka) virus in Valencia (Spain). *Acta Horticulturae* 309, 129–134.

MAPA, 1998. *Anuario de Estadística Agraria*. Ministerio de Agricultura Pesca y Alimentación, Madrid, Spain, 200 pp.

Marenaud C. and M. Yürektürk, 1974. Problèmes posés par la détection du virus de la sharka. *Pomologie Française* 16, 207–214.

Martínez-Gómez P. and F. Dicenta, 1999. Susceptibility of Spanish apricot cultivars to sharka (Plum pox potyvirus). *Phytopathologia Mediterranea* 38, 122–125.

Martínez-Gómez P. and F. Dicenta, 2001. Distribution of coat protein and nucleic acid of *Plum pox virus* (PPV) in seedlings of peach rootstock GF305 and apricot cv. Real Fino. *Phytopathologia Mediterranea* 40, 157–164.

Morvan G., 1988. Travaux de recherche et politique de lutte contre la sharka. *Compte Rendu de la Conference Internationale des Maladies des Plantes,* Bordeaux, France, 1137–1144.

Németh M., 1994. History and importance of plum pox in stone-fruit production. *EPPO Bulletin* 24, 525–537.

Pelet F. and R. Bovey, 1968. Les symptômes de la sharka sur les pruniers, pruneautiers, abricotiers et pêchers. *Agriculture Romande* 7, 80–84.

Reyes F., M.A. Reyes, P. Sepúlveda, J.J. López-Moya and H. Prieto, 2001. New insights of *Plum pox virus* in Chile. *Acta Horticulturae* 550, 135–140.

Roy A.S. and I.M. Smith 1994. Plum Pox situation in Europe. *EPPO Bulletin* 24, 515–523.

Sutula C.L., J.M. Gillet, S.M. Morrissey and D.C. Ramsdell, 1986. Interpreting ELISA data and establishing the positive-negative threshold. *Plant Disease* 70, 722–726.

Varveri C., D. Dimou and B. Di Terlizzi, 1999. Preliminary data of monitoring of natural spread of sharka in commercial orchards in Greece. *Acta Horticulturae* 488, 793–796.

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