Entomologia Generalis, p. 001–014
Stuttgart, Published online April 2014

Fast Track Article

Potential vectors of *Plum pox virus* in the Eastern Mediterranean Region of Turkey

Kamuran Kaya[1], Mona Gazel[1], Çiğdem Ulubaş Serçe[1], Eminur Elçi[1], Feza Can Cengiz[1], Mariano Cambra[2] & Kadriye Çağlayan[1]

[1]Mustafa Kemal University, Faculty of Agriculture, Plant Protection Department, 31034 Hatay, Turkey.
[2]Laboratorio de Virología e Inmunología, Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Ctra. Moncada-Náquera km 5, 46113 Moncada, Valencia, Spain.

With 3 figures and 4 tables

Abstract: Although *Plum pox virus* (PPV) was first detected in Turkey 44 years ago, the virus is present in a rather limited number of trees. Our recent studies on PPV incidence showed that PPV was introduced rapidly in PPV-free regions and that there are no data available about the role of aphid species and *Prunus* rootstocks on these new infections. In this study the epidemiological aspect of PPV was studied in Antakya-Hatay, located in the Eastern Mediterranean region of Turkey where PPV was first detected in 2011. The susceptibility of different *Prunus* rootstocks to PPV was evaluated in an established experimental plot next to a PPV-infected nectarine orchard. Aphid populations were monitored in 2011 and 2012 from the last week of April to the middle of June by the sticky-plant method in both the experimental plot (EP) and the surrounding infected nectarine orchard (SNO). Regularly collected plant samples and aphids were individually tested by DASI-ELISA and squash real-time RT-PCR, respectively. The highest aphid population densities were observed at the end of May in both years. The most abundant aphid species were *Aphis gossypii* and *A. spiraecola* both in EP and SNO in both years. The percentage of PPV-viruliferous *Myzus persicae*, *A. fabae*, *A. gosypii*, *A. spiraecola*, *Hyalopterus pruni*, *Macrosiphon euphorbiae* and *A. craccivora* as estimated by squash real-time RT-PCR were 39.47%, 25.00%, 24.56%, 22.60%, 22.22%, 20.00% and 8.00%, respectively. The percentages of viruliferous aphids collected from SNO were 12.5% in *A. spiraecola*, 12.42% in *A. gossypii* and 11.11% in *H. pruni*. At the end of 2012, three Myrobolan 29C and two Adesoto 101 rootstocks were found infected by PPV. Molecular characterization studies showed that PPV-M was the strain present in both the originally infected nectarine plot and the Myrobolan 29C rootstocks.

Keywords: Viruliferous aphids, sharka disease, epidemiology, rootstock susceptibility, natural

*Corresponding author: kayakamuran@gmail.com

www.schweizerbart.de
DOI: 10.1127/0171-8177/2014/00019
0171-8177/00019 $ 0.00

1 Introduction

Plum pox virus (PPV), the causal agent of sharka disease, is one of the most important pathogens threatening stone fruit cultivation. PPV belongs to the *Potyviridae* and is naturally transmitted by aphids (Homoptera: Aphididae). It is transmitted in a non-persistent manner, which means that aphids acquire the virus from infected plants within a few seconds and are able to transmit the virus immediately after acquisition (Shukla et al. 1994). Non-colonizing species are as efficient for virus spread as are aphid species that colonize *Prunus* spp. because brief feeding on a *Prunus* tree is sufficient for virus uptake and subsequent infection of healthy plants (Avinent et al. 1994). Today, about 20 aphid species are known to transmit PPV (Levy et al. 2000) and many of them are likely to occur in Turkey (Yumruktepe & Uygun 1994, Hazir et al. 2011). The efficiency of transmission is dependent on the virus strain, host cultivar, age of the host cultivar, aphid species, and season of year. The most important PPV-vectoring aphid species reported from several countries are *Myzus persicae* (Sulzer), *Aphis spiraecola* Patch, *Brachycaudus cardui* (L.), *B. helichrysi* (Kaltenbach), and *Phorodon humuli* (Schrank). Reports vary from country to country but natural virus transmission is commonly low in July and August but high in spring and autumn. Spring flights of *A. spiraecola, B. helichrysi, M. persicae* and *P. humuli* are the most important for virus transmission within and between orchards. Aphids were found to transmit PPV within a radius of 100 to 120 m of the source plants, but they have also been shown to carry the virus on their stylets for several kilometers if starved during flight (Levy et al. 2000).

PPV infections in Turkey have been known since 1968 but the disease was not common, except in apricot and plum trees in home gardens and ornamental parks in restricted areas. The presence of PPV in Turkey was first reported in the Marmara and Central Anatolia regions (Sahtiyanci 1969, Kurçman 1973). New PPV outbreaks were then recorded in the Aegean region (İzmir) (Gümüş et al. 2004). After 2006, the virus was reported in the Mediterranean region, which used to be completely free of Sharka disease. The first infection in this region was reported in the western part of the Mediterranean region of Turkey (Isparta-Egirdir) (Candresse et al. 2007) and then infections were commonly detected in apricot, plum, peach and nectarines in the eastern Mediterranean region of Turkey (Koç & Baloğlu 2006).

There are numerous PPV isolates differing in biological and epidemiological properties such as aggressiveness, aphid transmissibility and symptomatology. These differences have been serologically and molecularly documented, leading to the clustering of PPV into six serotypes or strains: PPV-D, PPV-M, PPV-EA, PPV-C, PPV-W and PPV-Rec (Candresse & Cambra 2006). The economic costs associated with the sharka disease caused by PPV involve not only direct losses in stone fruit production, eradication, compensatory measures and lost revenue, but also indirect costs including those from preventive measures such as quarantine, surveys, inspections, control nurseries, diagnostics and the impact on foreign and domestic trade (Cambra et al. 2006a).

In the years 2007 to 2010, extensive surveys were carried out by the Ministry of Agriculture in 56 of the 81 provinces of Turkey. A total of 5762 samples were

collected from almond, apricot, mahaleb cherry, nectarine, plum, peach, sweet and sour cherry and tested for PPV by biological indexing, DAS-ELISA and RT-PCR. Among these samples 222 plants were found to be infected with PPV (Akbaş et al. 2011). Until now, there are no detailed studies about the species composition and population dynamics of aphids in PPV-infected stone fruit orchards in Turkey. Therefore, the aims of this study were to investigate the species composition and population densities of potential PPV vectoring aphids, the percentage of viruliferous aphids and the reaction of several rootstocks to natural PPV infection.

2 Material and Methods

2.1 Plant material and experimental nursery plots

An experimental nursery plot was established in Antakya-Hatay, located in the Eastern Mediterranean Region in 2011 and plants were observed for three consecutive years. The susceptibility to natural PPV infection was evaluated in the most widely used *Prunus* rootstocks in Turkey and Europe such as Myrobolan 29C (*P. cerasifera*), Garnem (*P. dulcis* × ((*P. persica* × *Prunus davidiana*) × *P. persica*)), GF677 (*Prunus amygdalus* × *P. persica*), Docera 6 (*P. domestica* × *P. cerasifera*), Greenpac ((*P. persica* × *P. davidiana*) × (*P. dulcis* × *P. persica*) and Adesoto 101 (*Prunus insititia*). Two hundred plants of each rootstock, PPV-free certified, were planted next to a naturally PPV-infected nectarine orchard, serving as main inoculum source. The plots were planted in two rows parallel to the inoculum source by using a longitudinal design of 20 groups or replicates with 10 plants in each group (20 cm apart). The groups were randomly distributed. The experimental field was grown under standard nursery practices without any phytosanitary treatment. The nearest surrounding nectarine orchard (SNO), which was PPV-infected and 150 m away from the experimental plot, was also evaluated for PPV dissemination by aphids.

2.2 Monitoring of PPV spread

The main inoculum source, the nectarine orchard, was tested by DASI-ELISA in order to estimate PPV incidence before the experimental plot establishment. The rootstocks in the experimental plot were also individually tested for PPV absence at the beginning of plot establishment in late spring of 2010. Further testing was performed in spring and autumn of 2011 and 2012, respectively. Rootstock plants were regularly checked for PPV symptoms and individually sampled by collecting four fully expanded leaves from different parts of the canopy of each individual rootstock in both spring and autumn. Serological assays for PPV detection were performed by DASI-ELISA using the 5B-IVIA (Cambra et al. 1994) monoclonal antibody kit (Plant Print Diagnostics, Spain), following the EPPO (2004) protocol for PPV detection.

2.3 Molecular characterization of PPV isolates

Samples from one PPV-positive Myrobolan 29C plant from the experimental plot and also four randomly selected nectarine plants from the naturally infected orchard were analyzed for PPV strain identification. Leaf materials exhibiting PPV symptoms were picked and RNA extractions were performed using a LiCl method (Spiegel et al. 1996). A 100-mg sample of fresh material was homogenized in 5 volumes of LiCl buffer and the nucleic acids were extracted using 6 M potassium acetate (pH 6.5) and isopropanol precipitation. The pellet was suspended in 50 µl sterile water. Four µl of RNA were used for cDNA synthesis using a reverse transcription kit (MBI Fermentas, Finland). PCR was performed using *Plum pox virus* universal primer pairs amplifying 745 bp fragments of 5' terminal end of NIb and 3' end of CP: NCUniFor 5' – GAGGCAATTTGTGCTTCAATGG – 3' and NCUniRev 5' – CGCTTAACTCCTTCATACCAAG – 3' (Predajna et al. 2012). Annealing temperature was 53 °C for 1 min. DNA fragment amplified from PCR was directly sequenced and analyzed using "The Basic Local Alignment Search Tool (BLAST)" option of The National Center for Biotechnology Information (NCBI) web site http://blast.ncbi.nlm.nih.gov/Blast.cgi. Multiple alignments of the nucleotide sequences were performed using BioEdit (Hall 1999). The alignments were used to reconstruct phylogenetic trees using the neighbor-joining method with nucleotide identity distances, using the Mega 5.0 program (Tamura et al. 2011). Bootstrap analyses with 1000 replicates were performed to estimate the support for inferred phylogenies.

2.4 Monitoring of aphid species and detection of viruliferous aphids

Adult winged aphids visiting both the rootstock plants in the experimental plot and plants in the surrounding nectarine orchard (SNO) were monitored by the sticky-plant method (Avinent et al. 1993, Marroquín et al. 2004) during spring 2011 and 2012. Three and five sticky shoots were collected each week from each rootstock species and SNO, respectively, and new shoots were sprayed for the next collections. The collected sticky shoots were processed according to Capote et al. (2008) to identify aphid species and estimate their numbers. All identified individuals of the aphid species caught in the field from sticky rootstocks and SNO were used to estimate the number and percentage of PPV-viruliferous aphids visiting the experimental nurseries. Aphids were squashed individually on nylon membranes (Roche), using the rounded bottom of an Eppendorf tube to ensure complete crushing of each aphid. RNA was extracted from each squashed aphid individual using 100 µL buffer (0.1 M glycine, 0.05 M NaCl, 1 mM ethylenediaminetetraaceticacid (EDTA)) (Osman & Rowhani 2006) and analyzed by squash real-time RT-PCR (Olmos et al. 2005). Membranes without aphid squash were used for negative controls.

3 Results

3.1 Evaluation of the reaction of different *Prunus* rootstocks to natural PPV infection

All plants of the surrounding nectarine orchard (167 plants), serving as the main inoculum source, were tested by DASI-ELISA before experimental plot establishment and PPV-infection rate was found as 52.69%. One year after planting, the individual rootstocks in the experimental plot were also tested by DASI-ELISA in both spring (April) and autumn (September) of 2011 and 2012 and also spring of 2013. After the first year of cultivation, only one and the following year an additional Myrobolan 29C individual were found to be infected by PPV (Fig. 1). When all rootstocks were tested again in spring of 2013, five plants (three Myrobolan 29C and two Adesoto 101) tested positive for PPV. The main symptoms on Myrobolan 29C were vein clearing and chlorosis between the veins. No obvious symptoms were observed on Adesoto 101, which was found infected in spring of 2013. Due to quarantine regulation in Turkey, the PPV-infected nectarine orchard, serving as main inoculum source, was eradicated one year after experimental plot establishment.

Fig. 1. *Plum pox virus* (PPV) symptoms on a naturally infected Myrobalan 29 C *Prunus* rootstock in the experimental plot in Antakya-Hatay.

3.2 Characterization of PPV isolates

The PPV isolates obtained from the Myrobolan 29C plants from the experimental plot and four randomly selected plants from the naturally infected nectarine orchard were sequenced and analyzed. The sequences of Myrobolan 29C indicated 98% identity with the PPV isolates of N1 (Greece), SK68 (Hungary) and VAR2 (Slovakia) of PPV-M strains from the NCBI gene bank (accession numbers FJ361234, M92280 and AY324837, respectively). The nucleotide sequence identity of Myrobolan 29C from the experimental plot and four isolates from the nectarine orchard was greater than 99.7%. In the phylogenetic analysis all sequenced isolates clustered in the PPV-M strain clade (Fig. 2).

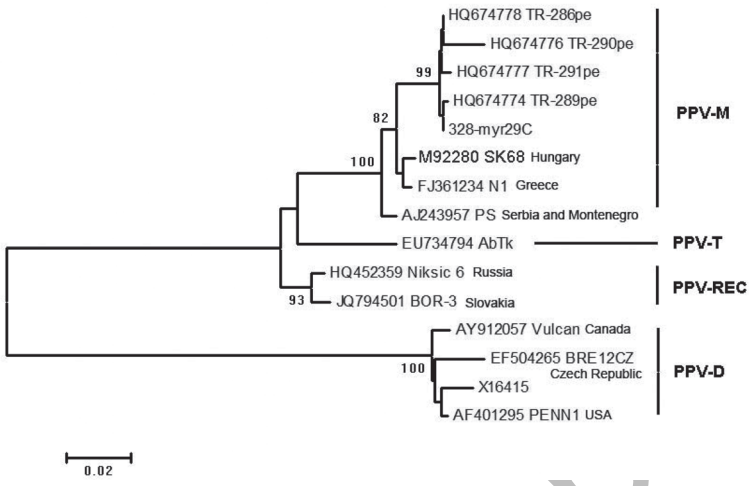

Fig. 2. Phylogenetic analysis of *Plum pox virus* isolates from Myrobolan 29C (328-myr29C) *Prunus* rootstocks and four nectarine plants (TR-286pe, TR-290pe, TR-291pe, TR-289pe). The reference sequences come from NCBI Genbank, the first capital letters are accession numbers followed by isolate names. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

3.3 Aphid occurrence and number of viruliferous aphids present in experimental plots

The aphid species landing on the rootstock plants of the experimental plot were identified in spring (from April to June) of 2011 and 2012 (Table 1). In both years, *Aphis gossypii* Glover and *A. spiraecola* were the most common aphid species. *A. gossypii* was most frequent in 2011 while *A. spiraecola* was most frequent in 2012.

The aphid community in the experimental plot began to increase in the second week of May and decreased in the first week of June (Fig. 3). The total aphid community reached its peak at the beginning of June (June 1) in 2011 and in mid-May (May 17) in 2012.

Among all collected aphid species, the highest percentage of PPV-viruliferous individuals was detected in *Myzus persicae* (39.47%) by squash real-time RT-PCR. This species was followed by 22–25% viruliferous individuals in *A. fabae* Scopoli, *A. gossypii*, *A.* and *Hyalopterus pruni* (Geoffroy) (Table 2). Regarding rootstocks, the highest percentage of viruliferous aphids was found on Myrobolan 29C (41.66%) followed by Adesoto 101, Greenpac, Garnem, Docera 6 and GF 677.

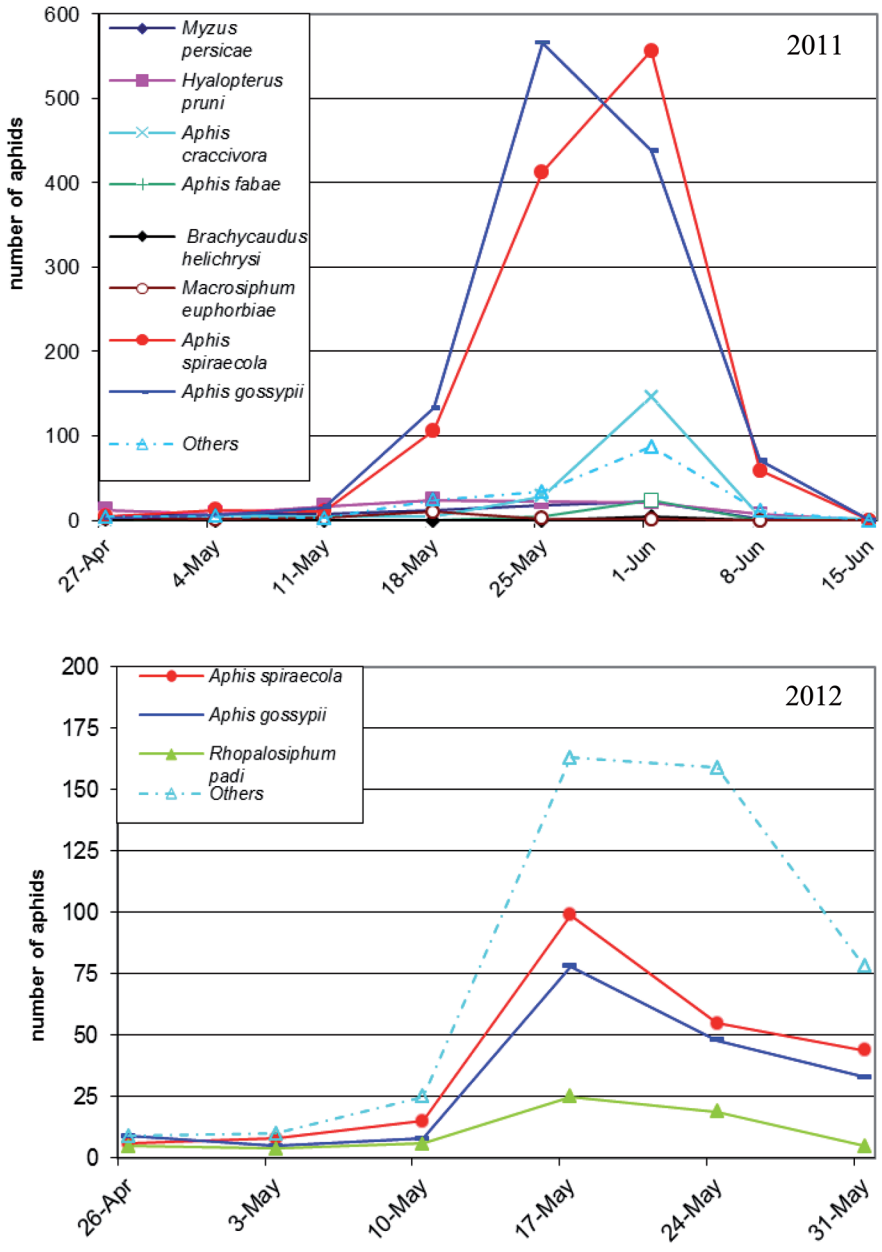

Fig. 3. Mean number of aphid species caught each week using the sticky-plant method in the experimental plot of *Prunus* rootstocks in Antakya-Hatay in 2011 and 2012.

Table 1. Occurrence of *Plum pox virus*-vectoring aphid species in the experimental plot in Antakya-Hatay. Number and percentage of individuals caught in 2011 and 2012 (April–June).

Aphid species	2011		2012	
	Number	%	Number	%
Aphis gossypii	1232	41.10	181	19.75
A. spiraecola	1162	38.77	227	24.78
A. craccivora	196	6.53	–	–
Hyalopterus pruni	111	3.70	–	–
Myzus persicae	73	2.43	–	–
A. fabae	30	1.00	–	–
Macrosiphum euphorbiae	20	0.66	–	
Brachycaudus helichrysi	5	0.16	–	–
Rhophalosiphum padi	–	–	64	6.98
Others	168	5.60	444	48.47
Total	2997		916	

In addition to sampling in the experimental plot (EP), aphids were collected from the surrounding nectarine orchard (SNO) using the same method and on the same dates as in EP (Table 3). Similar to EP and in both years 2011 and 2012, in SNO the two most common aphid species were *A. gossypii* and *A. spiraecola*.

Table 3. List of aphid species collected from the nectarine orchard surrounding the experimental plot in Antakya-Hatay in 2011 and 2012.

Aphid species	2011		2012	
	Number	%	Number	%
Aphis gossypii	169	53.99	29	19.33
A. spiraecola	103	32.90	35	23.33
Hyalopterus pruni	10	3.19	–	–
Myzus persicae	5	1.59	–	–
Aphis craccivora	5	1.59	9	6.00
Macrosiphum euphorbiae	3	0.95	–	–
Rhopalosiphum padi	–	–	23	15.33
Others	18	5.75	54	36.00
Total	313		150	

Table 2. The number of viruliferous aphid / total aphid species collected from different rootstocks in the experimental plot in Antakya-Hatay in 2011.

Aphid species	Rootstock						Total
	Garnem	Docera	Myrobalan	GF 677	Greenpac	Adesoto	
Myzus persicae	3/8	8/16	1/5	1/5	1/3	1/1	15/38 (39.47%)
Aphis fabae	0	1/8	0	2/4	0	0	3/12 (25.00%)
A. gossypii	7/38	20/141	15/28	2/9	2/15	25/58	71/289 (24.56%)
A. spiraecola	6/39	34/157	12/27	0/9	13/52	1/8	66/292 (22.60%)
Hyalopterus pruni	1/2	1/7	0/6	2/3	0/1	2/8	6/27 (22.22%)
Macrosiphum euphorbiae	0/3	0	1/2	1/5	0	0	2/10 (20.00%)
A. craccivora	1/6	1/22	0	1/19	0/1	1/2	4/50 (8.00%)
Others	1/3	3/19	1/4	0/1	1/2	1/3	7/32 (21.87%)
Total	19/99 (19.19%)	68/370 (18.37%)	30/72 (41.66%)	9/55 (16.36%)	17/74 (22.97%)	31/80 (38.75%)	174/750 (23.20%)

The percentage of detection of viral RNA in individuals of the most abundant PPV-vectoring species ranged from 12.5% for *A. spiraecola* to 11.1% for *H. pruni* (Table 4). In total, 11.85% of the aphid individuals visiting SNO in the period studied were PPV-viruliferous.

Table 4. The number of viruliferous / total individuals per aphid species collected from the surrounding nectarine orchard (SNO) of the experimental plot in Antakya-Hatay in 2011.

	A. gossypii	*A. spiraecola*	*H. pruni*	*M. persicae*	*A. craccivora*	*M. euphorbiae*	**Total**
Viruliferous/ total number	21/169	10/80	1/9	0/4	0/5	0/3	32/270
% viruliferous	12.42	12.5	11.11	–	–	–	11.85

4 Discussion

In the present study nine aphid species that are known as PPV vectoring species were identified in both an experimental plot (EP), consisting of various *Prunus* rootstocks, and a surrounding nectarine orchard (SNO) in Antakya-Hatay. This number of aphid species is nearly half the number of currently known aphid species vectoring PPV (Levy et al. 2000). The most numerous migrant aphid trapped in Antakya-Hatay was *A. gossypii*, the cotton aphid, which has been reported as a minor PPV vector in Spain (Avinent et al. 1994) and France (Labonne et al. 1994). Because Antakya is an important cotton growing region in Turkey, a high population of this aphid was expected but, for the first time, a relatively high percentage of viruliferous *A. gossypii* (24.56%) was reported. The second and third most numerous species trapped in the experimental plot were *A. spiraecola* (38.77% of all aphids trapped in 2011) and *A. craccivora* Koch (6.53% of all aphids trapped in 2011) both of which are polyphagous, but only *A. craccivora* does occasionally colonize *Prunus* spp. (Levy et al. 2000). Due to its frequency in the aphid community and high percentage of viruliferous individuals (22.66%), *A. spiraecola* seems to be an efficient vector of PPV in the Mediterranean region of Turkey, as previously reported by Wallis et al. (2005) and Cambra et al. (2006b). This aphid species was also frequently found in other Mediterranean countries of Western Europe (Avinent et al. 1991, Labonne et al. 1994). Recently it has been shown that *A. spiraecola* was the most abundant aphid species in orchards of Valencia, Spain with an average PPV infection rate of 30.37% when inoculum pressure was high (Vidal et al. 2012). Migrant aphid species that do not colonize *Prunus* spp. can also be important for PPV transmission because extremely high populations of specific migrant species on other trees or shrubs surrounding the orchards may move into stone fruit orchards and transmit the virus during short feeding periods (Wijkamp & Gaag 2011). Another possible PPV vector might be *Hyalopterus pruni*, which accounted for 3.70% of the total number of aphids caught in 2011, and had a reasonably high infection rate by

PPV (22.22%). This aphid species can colonize *Prunus* spp. and was reported as an important PPV vector in Eastern Europe (Zsuzsa et al. 1997, Isac et al. 1998). However, a population of *H. pruni* in France was unable to transmit PPV in replicated tests using three different PPV isolates (Labonne et al. 1995). There is also one report from Turkey about the transmission of PPV from naturally infected *Prunus* spp. to GF305 by viruliferous *H. pruni* (Elibüyük 2003), but no data is available about transmission efficiency. Although the frequency of *M. persicae* in the population was found to be very low (2.43%), this aphid had the highest infection rate (39.47%) among the collected aphids. *M. persicae* can colonize *Prunus* trees and is considered an efficient vector of PPV (Labonne et al. 1995, Llácer & Cambra 1998). It was already shown that *M. persicae* able to acquire PPV-M from its primary host plant, *Prunus persica*, and transmit it to secondary host plants and vice versa (Manachini et al. 2004, 2007). In our study, PPV-M was also successfully transmitted from the infected nectarine orchard to the Myrobolan 29C and Adesoto 101 rootstocks, but the transmission of PPV was relatively low in the first year after establishment of the experimental plot (0.083%) but increased to 0.643% after three years. This low PPV infection rate in even sensitive rootstocks might be due to the eradication of the PPV infected nectarine orchard (the main inoculum source) one year after establishment of the experimental rootstock plantation (EP) and the long distance (150 m) between the surrounding PPV infected nectarine orchard (SNO) and the EP. It was shown that natural transmission of PPV-M may occur over distances up to 150 m (Capote et al. 2010). Under high inoculum pressure, PPV can spread rapidly via natural transmission and disease incidence starting at less than 10% can reach up to 100% within five years (Gottwald et al. 1995, Varveri 2006).

In our study, PPV was naturally transmitted to the experimental *Prunus* orchard, which was PPV-free when it was established. The nearest SNO, which was 150 m away from the experimental plot, was also evaluated for PPV infection and aphid occurrence. PPV was detected in aphids caught in the commercial SNO in 2011, after establishment of the experimental plot, with the most common aphid species *A. gossypii* and *A. spiraecola*. The percentage of viruliferous individuals of all aphid species was 23.20% and 11.85% in EP and SNO, respectively. The PPV isolates sequenced from the experimental plot (EP) and the SNO, serving as the main inoculum source in our study, were almost perfectly identical, despite the ability of plant RNA viruses to rapidly evolve and change (Moury et al. 2006). There have been several researches on PPV diversity in a single tree. For example, a PPV-M isolate could evolve into several distinct populations 15 years after inoculation of a peach seedling with PPV-M (Jridi et al. 2006). Altogether, our study shows that in stone fruit plantations in the Antakya-Hatay region of Turkey efficient PPV vectoring aphid species and a high percentage of viruliferous aphids occur. Although a rootstock susceptibility experiment is in progress, tentative results suggest that Myrobolan 29C seems to be more sensitive to natural PPV-M infection than other rootstocks. Vidal et al. (2012) reported that Cadaman, Garnem and GF-677 are less susceptible PPV rootstocks and, together with environmentally friendly treatments such as use of mineral oils against aphids, constitute an integrated strategy to reduce PPV spread. Garnem and GF-677 also remained uninfected in our experiment for two years, and, might therefore be favorable rootstocks for stone fruit production in the Antakya-Hatay region.

Acknowledgements

The research leading to these results received funding from the European Community's Seven Framework Programme (FP7/2007-2013) under Grant Agreement n° 204429, SharCo project. The authors would like to thank Prof. Dr. Anne Frary from Izmir Institute of Technology for her kind help for English editing.

References

Akbaş, B., Değirmenci, K., Çiftçi, O., Kaya, A., Yurtmen, M., Uzunoğullari, N., Çelik, N. & Türkölmez, Ş. (2011): Update on *Plum pox virus* distribution in Turkey. – Phytopathol. Mediterr. **50:** 75−83.

Avinent, L., Hermoso De Mendoza, A. & Llácer, G. (1991): Comparison of traps for capture of alate aphids (Homoptera, Aphididae) in apricot tree orchards. – Agronomie **11:** 613–618.

Avinent, L., Hermoso De Mendoza, A. & Llácer, G. (1993): Comparison of sampling methods to evaluate aphid populations (Homoptera, Aphidinea) alighting on apricot trees. – Agronomie **13:** 609–613.

Avinent, L., Hermoso De Mendoza, A. & Llácer, G. (1994): Transmission of PPV in Spain. – EPPO Bull. **24:** 339–674.

Cambra, M., Asensio, M., Gorris, Mt., Perez, E., Camarasa, E., Garcia, J.A., Moya, J.J., Lopez-Abella, D., Vela, C. & Sanz, A. (1994): Detection *Plum pox potyvirus* using monoclonal antibodies to structural and non-structural proteins. – EPPO Bull. **24:** 569–577.

Cambra, M., Capote, N., Myrta, A. & Llácer, G. (2006a): *Plum pox virus* and estimated cost associated with Sharka disease. – EPPO Bull. **32:** 202–204.

Cambra, M., Capote, N., Cambra, M.A., Llácer, G., Botella, P. & López, A. (2006b): Epidemiology of sharka disease in Spain. – EPPO Bull. **36:** 271–275.

Candresse, T. & Cambra, M. (2006): Causal agent of Sharka disease, historical perspective and current status of *Plum pox virus* strains. – EPPO Bull. **36:** 239–246.

Candresse, T., Svanella-Dumas, L., Gentit, P., Çağlayan, K. & Çevik, B. (2007): First report of the presence of *Plum pox potyvirus* Rec strain in Turkey. – Plant Dis. **91:** 331.

Capote, N., Bertolini, E., Martínez, M.C., Olmos, A., Gorris, M.T. & Cambra, M. (2008): Spot real-time RT-PCR: A method for direct detection of *Plum pox virus* avoiding RNA extraction. – Acta Hortic **781:** 215–220.

Capote, N., Cambra, M.A., Botella, P., Gorris, M.T., Martínez, M.C., López-Quílez, A. & Cambra, M. (2010): Detection, characterization, epidemiology and eradication of *Plum pox virus* Marcus type in Spain. – J. Plant Pathol. **92:** 619–628.

Elibüyük, İ.Ö. (2003): Natural spread of *Plum pox virus* in Ankara, Turkey. – J. Phytopathol. **151:** 617–619.

EPPO (2004): Diagnostic protocol for regulated pests. *Plum pox potyvirus*. – OEPP Bull./EPPO Bull. **34:** 247–256.

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

Gümüş, M., Al-Rwahnih, M. & Myrtha, A. (2004): First report of apricot latent virus in Turkey. – J. Plant Pathol. **86:** 92.

Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucl. Acids Symp. Ser. **41:** 95–98.

Hazir, A., Yurtmen, M., Özdemir, I. & Aksoy, E. (2011): Aphid (Hemiptera: Aphididae) species composition and potential aphid vectors of *Plum pox virus* in stone fruit orchards and on weeds

in the East Mediterranean region. – Proc. IVth Plant Prot. Congr. Turkey, 28–30 June 2011, Kahramanmaraş, pp. 226 (in Turkish abstr.).

Isac, M., Preda, S. & Marcu, M. (1998): Aphid species –Vectors of *Plum pox virus*. – Acta Virol. **42:** 233–234.

Jridi, C., Martin, J.F., Marie Jeanne, V., Labonne, G. & Blanc, S. (2006): Viral Populations Differentiate and Evolve Independently in a Single Perennial Host Plant. – J. Virol. **80** (5): 2349–2357.

Koç, G. & Baloğlu, S. (2006): First report of sharka in the Çukurova region of Turkey. – J. Plant Pathol. Suppl. **88** (3): 68.

Kurçman, S. (1973): Nachweis des Sharka-Virus an Aprikosen- und Pflaumenbäumen in Ankara. – J. Turkish Phytopathol. **2:** 124–129.

Labonne, G., Lauriaut, F., Yvon, M. & Quiot, J.B. (1994): Dissemination du *Plum pox potyvirus* par les pucerons: analyse des vecteurs potentiels du virus dans un verger d'abricotiers. – EPPO Bull. **24:** 681–690.

Labonne, G., Yvon, M., Quiot, J.B., Avinent, L. & Llacer, G. (1995): Aphids as potential vectors of *Plum pox virus*: comparison of methods of testing and epidemiological consequences. – Acta Hortic **386:** 207–218.

Llácer, G. & Cambra, M. (1998): Thirteen years of sharka disease in Valencia, Spain. – Acta Hortic **472:** 379–384.

Levy, L., Damsteegt, V., Scorza, R. & Kolber, M. (2000): *Plum Pox Potyvirus* Disease of Stone Fruits. – APSnet Features, online: doi 10.1094/APSnetFeature-2000-0300.

Manachini, B., Casati, P., Aliverti, I. & Cinanni, L, (2004): Transmission of PPV-M to *Prunus persicae* by *Brachycaudus schwartzi* and *Phorodon humuli* (Hemiptera: Aphididae). – J. Appl. Entomol. **128:** 677–688.

Manachini, B., Casati, P., Cinanni, L. & Bianco, P. (2007): Role of *Myzus persicae* (Hemiptera: Aphididae) and its secondary hosts in *Plum pox virus* propagation. – J. Econ. Entomol. **100:** 1047–1052.

Marroquin, C., Olmos, A., Gorris, Mt., Bertolini, E., Martinez, Mc., Carbonell, E., Hermoso De Mendoza, A. & Cambra, M. (2004): Estimation of the number of aphids carrying *Citrus tristeza virus* that visit adult citrus trees. – Virus Res. **100:** 101–108.

Moury, B., Desbiez, C., Jacquemond, M. & Lecoq, H. (2006): Genetic diversity of plant virus populations: towards hypothesis testing in molecular epidemiology. – Adv. Virus Res. **67:** 49–87.

Olmos, A., Bertolini, E., Gil, M. & Cambra, M. (2005): Real-time assay for quantitative detection of non-persistently transmitted *Plum pox virus* RNA targets in single aphids. – J. Virol. Methods **128:** 151–155.

Osman, F. & Rowhani, A. (2006): Application of a spotting sample preparation technique for the detection of pathogens in woody plants by RT-PCR and real-time PCR (TaqMan). – J. Virol. Methods **133:** 130–136.

Predajňa, L., Šubr, Z., Candresse, T. & Glasa, N. (2012): Evaluation of the genetic diversity of *Plum pox virus* in a single plum tree. – Virus Res. **167** (1): 112–117.

Sahtiyanci, Ş. (1969): Virus de la sharka chez le prunier. – Bull. Phytosanitaire FAO **17:** 69.

Shukla, D.D., Ward, C.W. & Brunt, A.A. (1994): The Potyviridae. – C.A.B. International, Wallington, Oxford.

Spiegel, S., Scott, S.W., Bowman-Vance, V., Tam, Y., Galiakparov, N.N. & Rosner, A. (1996): Improved detection of *prunus necrotic ringspot virus* by polymerase chain reaction. – Eur. J. Plant Pathol. **102:** 681–685.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011): MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. – Mol. Biol. Evol. **28:** 2731–2739.

Varveri, C. (2006): Epidemiology of *Plum pox virus* strain M in Greece. – EPPO Bull. **36:** 276–278.

Vidal, E., Moreno, A., Bertolini, E. & Cambra, M. (2012): Estimation of the accuracy of two diagnostic methods for the detection of *Plum pox virus* in nursery blocks by latent class models. – Plant Pathol. **61:** 413–422.

Wallis, C.M., Fleischer, S., Luster, J.D. & Gildow, F.E. (2005): Aphid (Hemiptera: Aphididae) species composition and potential aphid vectors of *Plum pox virus* in Pennsylvania peach orchards. – J. Econ. Entomol. **98:** 1441–1450.

Wijkamp, I. & Jan Van Der Gaag, D. (2011): Pest risk analyses for *Plum pox virus* (PPV). – Plant Protect. Serv., pp. 57, Wageningen.

Yumruktepe, R. & Uygun, N. (1994): Determination of aphid species (Homoptera: Aphididae) and their natural enemies in Citrus orchards in Eastern Mediterranean region. – Proc. IIIrd Nat. Congr. Biol. Contr., 25–28 January 1994, 1–12, İzmir.

Zsuzsa, B., Dalma, P. & Richard, G. (1997): Flight and transmission activity of PPV vector aphids. – J. Aphidol. **11:** 21–27.