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Susceptibility of different prunus rootstocks to natural infection of plum pox virus-Turkey (PPV-T) in Central Anatolia

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ABSTRACT

Sharka is the most damaging viral disease of *Prunus* spp. and the causative agent is plum pox virus (PPV). PPV is widespread and poses a serious problem in stone fruit crops in Turkey. A unique strain of PPV has so far been identified in Turkey and called as PPV-Turkey (PPV-T). The aims of the present study were to describe the efficiency of PPV-T transmission via aphids and to identify the susceptibility of different *Prunus* rootstocks against PPV-T strain under natural inoculum in Kayseri province located Central Anatolia Region of Turkey. This experiment was established in a small residential orchard where PPV-T infected apricot and plum trees were detected. Five different *Prunus* rootstocks from which 200 individuals were planted in PPV-T infected orchard and experiment was designed in a randomized complete block desing with 10 replications per block. All rootstocks were regularly tested by DAS-ELISA and RT-PCR during 2015–2017. The results showed that the most susceptible rootstocks in terms of PPV-T infection were Nemaguard, Myrobalan 29B and Myrobalan 29C with the infection rate of 3.61%, 2.74% and 1.04%, respectively. On the other hand, GF677 and Garnem were never found infected with PPV-T. *Aphis gossypii*, *A. craccivora*, *A. spiraecola*, *Brachycaudus helichrysi*, *Hyalopterus pruni*, *Myzus persicae*, *Macrosiphum euphorbiae* were detected as most abundant aphid species in experimental plot. In 2015, the highest percentage of viruliferous aphids was found as *A. gossypii* (100%), followed by *B. helichrysi* (77.8%), *A. spiraecola* (55.72%), *M. persicae* (46.05%) and *H. pruni* (30.76%) whereas it was 100% for *A. gossypii* and *M. euphorbiae* followed by *A. spiraecola* (76.32%), *M. persicae* (73.1%), *H. pruni* (62.06%) and *B. helichrysi* (61.53%) in 2016 by squash real time RT-PCR. The present study showed that PPV-T was effectively transmitted by different aphid species in natural conditions in Kayseri district. It was concluded that Nemaguard and Myrobalan which were found to be the most sensitive rootstocks to PPV-T should not be preferred by growers as *Prunus* rootstock where PPV poses a high risk.

1. Introduction

Plum pox virus (PPV) is a plant virus (genus *Potyvirus*, family *Potyviridae*) and it causes Sharka disease, which is one of the most devastating viral diseases of stone fruits. (Porcel et al., 2018). It was described for the first time in Bulgaria in 1932 [1] and nowadays widespread worldwide, except in Australia, New Zealand, and South Africa. Sharka occurrence has serious agronomic, economic, and political consequences. The reduced fruit quality and premature fruit drop caused by sharka disease make stone fruit production very problematic and sparse local eradication programmes were not efficient enough to stop the continuous spread of PPV in the world [2]. A conservative estimate of the losses resulted in unmarketable apricot fruits is 0.6 million metric tonnes per production year. This would imply that 18 million metric tonnes have been lost in the last 30 years with an estimated value of 3.6 billion euros in the world. The global economic losses are estimated at hundreds of millions of euros annually, and millions of PPV-infected peaches, apricot, and plum trees have been destroyed to prevent the spread of the infection [2].

In 2018, approximately 45 million metric tons of stone fruit including peaches, nectarines, plums, apricots, and cherries were produced worldwide in 7.3 million hectares [3]. Global demand for stone fruit is getting increased due to its high nutritional value and desirable taste. The major exporters of stone fruit are South Africa and Chile in the

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southern hemisphere wheras Spain and Turkey are the largest exporters in the northern hemisphere [4,5]. In 2018, Turkey was in the top five largest stone fruit producer in the world with 5.91%, producing approximately 2.66 million tons [3]. The proportion of stone fruit production of Turkey in the world, which included mostly cherries (25.1%), apricots (19.5%), sour cherries (12%), peaches and nectarines (3.2%), and plums (2.4%) [3].

PPV was discovered for the first time in Turkey in 1968 [6]. Although PPV-D, PPV-M, Rec strains of PPV reported earlier in Turkey, a new strain called PPV-Turkey (PPV-T) was recently reported by Ulubas Serce et al. [53] and described as the most common strain in our country [7,8]. The main methods of controlling the disease are eradication of infected plants and breeding resistant crops that are not susceptible to the virus [9].

The use of PPV resistant or PPV tolerant *Prunus* rootstocks is an agronomic strategy to decrease the spread of the disease in nurseries and, therefore, in new orchards. PPV resistance has been reported in some *Prunus* rootstocks, such as BN 4Kr myrobalan plum [10], GF 677 (*Prunus dulcis* × *Prunus persica*), Myrobalan 29C (*Prunus cerasifera*) and L2 (*Prunus lannesiana*) [11]. However, the susceptibility of the most important *Prunus* rootstocks used in the stone fruit industry to natural PPV infection by viruliferous aphid species has not been well evaluated under open field conditions up to last years [12].

The rate of spread of the virus in the orchards depends on the distance between healthy trees and source of infection and the PPV transmission effectiveness depends on the sensitivity of culture and population density of aphids. But the main route of transmission of the virus occurs with the help of aphids [13,14]. The virus is spread locally by aphids in a non-persistent manner [12]. Once, introduction has occurred, the dissemination in the local place is for the action of more than 20 aphid species. These aphids can be natural vectors because they taste or feed on infected leaves, after they fly to other trees where they taste again or feed, spreading PPV between fruit trees [15,16]. Aphids were found to transmit PPV within a radius of 100–120 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 [17]. The most efficient aphid vectors of PPV have been reported as *M. persicae, A. gossipii* and *A. craccivora* [16,18]. PPV is vectored by aphids in a non-persistent manner and 'stylet borne' (carried at the stylet tips), transmitted to the new host plants through salivating during probing [19,54]. The maximum ability of PPV transmission by aphids was recorded at temperatures of 20–23 °C [13,14]. Transmission ability of PPV-T and PPV-M with aphids has been already demonstrated in Izmir (altitude of 30 m) and Hatay (altitude of 85 m), at altitudes close to sea level in the Aegean and Mediterranean regions in Turkey, respectively [21,22]. No studies have been conducted which montains might play an active role in PPV transmission in high altitude such as the Central Anatolian Region (altitude of Kayseri district: 1.054 m).

The main purposes of this study were to identify aphid species, responsible for transmission of PPV-T which is the most common strain in Kayseri district located in Central Anatolia Region of Turkey and study rootstock reactions to this PPV strain under natural inoculum conditions.

2. Material and methods

2.1. Experimental orchard

An experimental nursery plot was established in an orchard where PPV-T infected apricot and plum orchard were detected in previous studies [8,23] (Table 1). Five different one-year old and *in vitro* propagated rootstocks such as Nemaguard (*P. persicae* × *P. davidiana*), Myrobalan 29B and 29C (*P. cerasifera*), Garnem (*P. dulcis* × ((*P. persica* × *P. davidiana*) × *P. persica*))) and GF 677 (*P. amygdalus* × *P. persica*) were used for the experiment. From each rootstock 200 plants were planted, and experiment was designed in a randomized complete block desing with 10 replications per block. The plants were planted in two rows parallel to the inoculum source by using longitudinal design of 5 groups plants into each group (20 cm apart). The experimental field was managed according to standard nursery practices without any phytosanitary treatment.

Table 1
Natural incidence of Plum pox virus-T determined by DAS-ELISA and RT-PCR in different *Prunus* rootstocks in an experimental nursery plot in Kayseri province.

Name of the rootstocks	Number of infected/tested plants (2015)	Number of infected/tested plants (2016)	Number of infected/tested plants (2017)
Garnem	0/134	0/134	0/125*
GF 677	0/144	0/141	0/138
Myrobalan 29C	0/74	2/73	2/72
Myrobalan 29B	0/97	1/96	1/96
Nemaguard	0/192	6/166	6/170
Total plants	0/641	9/610	9/601

* Some rootstocks have dried up due to unsuitable weather conditions.

2.2. Monitoring of PPV spread

Serological tests were carried out one year after rootstocks planting, i.e., May of 2015–2017. Rootstocks were regularly checked for PPV symptoms and individually sampled by collecting four fully expanded leaves from different parts of the canopy. Serological assay for PPV detection was by DAS-ELISA based on the 5B-IVIA [24] monoclonal antibody kit (Bioreba, Switzerland), following the [25] protocol for PPV detection.

2.3. Molecular characterization of PPV isolates

PPV-positive samples obtained by DAS-ELISA in the experimental plot and six *Prunus* (apricot and plum) samples randomly selected in the adjacent infected orchard were analyzed for PPV strain identification in the second year of the monitoring. Leaves exhibiting PPV symptoms were collected, and RNA was extracted using a plant total RNA kit (Geneaid Plant Total RNA Mini Kit, Taiwan). Ten µl of RNA were used for cDNA synthesis. PCR was performed using PPV universal primer pairs that amplify 835 bp fragments PP3, (5′-TTATCTCCAGGARTTG-GAGC-3′) ve PCI, (5′-TTGAGTCAAATGGRACAGTTGG-3′) [26]. Cycling parameters were 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 52 °C for 45 s and 72 °C for 1 min, followed by 72 °C for 10 min. PCR amplicons were sequenced directly and analyzed using the basic local alignment search tool (BLAST). Multiple alignments of the nucleotide sequences of these isolates and reference isolates for each PPV strain obtained from GenBank were performed using BioEdit [27]. Cluster analysis was done with the Mega X program using the neighbor-joining method with nucleotide identity distances. 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

Aphid populations were monitored during a two-year period (2015–2016) using the sticky shoot method by using a spray (Soveurode aerosol, Scotts Biosystèmes, France) [28,29,50]. Winged aphid species were captured landing on the experimental plot each year during from spring to late summer 2015 and 2016. Three fully expanded leaves per each tree were sprayed, and aphids landing on sprayed leaves remained stuck to the leaves. After 7 days, leaves with captured insects were picked and put in jars, and three new leaves were sprayed with the adhesive. Back in the laboratory, jars with the leaves were filled with turpentine. The collected aphids were washed 2–3 times with soapy water to remove traces of turpentine. The aphids were preserved in 70% ethanol for later identification and counting. Three sticky shoots from

each rootstock species were collected each week and new shoots were sprayed for next collections. Collected shoots were processed to identify aphid species and estimate their numbers [30,31]. Identification of aphids was performed by using a stereomicroscope and keys for identification of alate aphids. Aphids were squashed individually on nylon membranes using the round bottom of an eppendorf tube to ensure complete disruption of each aphid. The nylon membranes with squashed aphids were stored in a dry place at room temperature until use. On the membranes 0.5% Triton X-100 was added, vortexed and incubated for 2 min at room temperature [32] and analyzed by squash real time RT-PCR [33].

Specific primers associated with the coat protein sequence of PPV (P241 primer, 5′- CGTTTATTTGGTTGGATGGAA-3′; P316D primer, 5′-GATTAACACACCAGCGGTGTG-3′; P316 M primer, 5′-GATTCACGTCACAGCGGTGTG-3′) and probe (5′-FAM-CGTCGGAACACAAGAAGAGGACACAGA-TAMRA-3′) [20] with reporter florasan dye (6-carboxy-fluorescein; FAM) and tagged at the 3′ end with quencher (6-carboxytetramethyl-rhodamine; TAMRA) [32,33]. Real-time RT-PCR master mix was prepared as suggested by the manufacturer (RNA Virus Master, version 08, Roche Diagnostics GmbH, Mannheim, Germany). The master mix included 0.4 μl enzyme, 4 μl reaction buffer, 0.5 μM F1 primer, 0.5 μM R1 primer, 0.5 μM R2 primer, 0.2 μM probe and 5 μl viral RNA. The total volume of prepared mixture was 20 μl. The PCR conditions were 1 cycle at 45 °C for 10 min, 1 cycle of 95 °C for 10 min, 45 cycles of 95 °C for 15 s, 60 °C for 45 s. Real-time RT-PCR results were conducted to generated CT (threshold cycle) values through the Software 4.1 of Roche 480 II (Roche Diagnostics GmbH, Mannheim, Germany).

3. Results

3.1. Evaluation of the susceptibility of different Prunus rootstocks to natural PPV-T infection by symptomological observation, DAS-ELISA, and RT-PCR

Individual rootstocks in the experimental plot were tested by DAS-ELISA in April and August of 2015 2016 and 2017. No PPV infected rootstock was detected in the first year of experiment (2015). The most susceptible rootstocks in terms of PPV-T infection rate were detected as Nemaguard, Myrobalan 29B and Myrobalan 29C with the infection rate of 3.61%, 2.74% and 1.04% by DAS-ELISA and RT-PCR in 2016, respectively. The other two rootstocks were PPV-negative during three years of observations (Table 1). In the experimental orchard among 18 trees (20–60 m away from experimental rootstocks) 6 were positive as inoculum source.

The most obvious symptoms on the PPV infected rootstocks were vein clearing and interveinal chlorosis (Fig. 1). Due to Turkish quarantine regulations, the PPV-infected orchard and all rootstocks were eradicated three years after the establishment of the experimental plots.

3.2. Characterization of PPV isolates

When the sequences of PCR amplicons obtained from nine PPV isolates were analyzed, 99% identity with the AnYnAp129, KrMrAp504 andP145 isolates of PPV-T (GenBank accession Nos. MF346257, MF346280 (Kayseri province of Turkey) and EU734802 (Ankara province of Turkey) was detected. The nucleotide sequence identity of the nine PPV isolates from the experimental plot (GenBank accession Nos. Myrobalan 29C (MW413829, MW413820), Myrobalan 29B (MW413821), Nemaguard (MW413816, MW413817, MW413818, MW413823, MW413824, MW413825) and of six additional isolates from the adjacent apricot and plum orchard (GenBank accession Nos. MW413822, MW413826, MW413827, MW413828, MW413829, MW413830) was detected as 99%. Phylogenetic trees of the PPV sequences were inferred the neighbor-joining (NJ) method implemented in Mega X and all the isolates obtained in this study found to be belonging to the strain. In the phylogenetic tree, all PPV-T isolates were grouped together in the first clade and closely related to PPV–An and some of the PPV-M strains. While PPV-Rec, PPV-D and PPV-EA isolates clustered together in another clade but in the same branch as PPV-T, cherry and Winona isolates (PPV-CR, PPV-C and PPV-W) were grouped together in the second branch (Fig. 2).

When the PPV-T isolates obtained in this study were phylogenetically analyzed together with other PPV-T isolates deposited in the GenBank, our isolates were grouped together with other PPV-T isolates obtained from the same geographic region (Central Anatolia) except one isolate (MF346280). All PPV-T Istanbul isolates grouped together in a different branch (Fig. 3).

3.3. Determination of aphid species and number of viruliferous aphids present in the experimental plot

The aphid species landing on the rootstocks in the experimental plot were identified during from spring to late summer (from May to August) of 2015 and 2016. A total of 1,170 individual aphids landing on sampled sticky leaves of the rootstocks in the experimental orchard were captured and identified. While 451 aphids were caught in 2015, 719 aphids were entraped in 2016. Collected aphids were morphologically characterized and *Aphis gossypii*, *A. craccrivora*, *A. spiraecola*, *Brachycaudus helichrysi*, *Hyalopterus pruni*, *Myzus persicae*, *Macrosiphum euphorbiae* were detected as most abundant species in the experimental plot. *A. spiraecola*, *H. pruni* and *M. persicae* were the most common aphids in both years, followed by *A. gossypii* and *B. helichrysi*. In both years, the total aphid number reached a peak at the end of June (16th of June in 2015 and 2016, 23rd of June in 2015 and 2016) (Fig. 4). The aphid population was found to be quite low in the first week of May,



Fig. 1. Vein clearing and chlorosis on the leaves of Nemaguard (left) and Myrobalan 29C (right) rootstocks in the experimental plot due to Plum pox virus (T strain) infection.

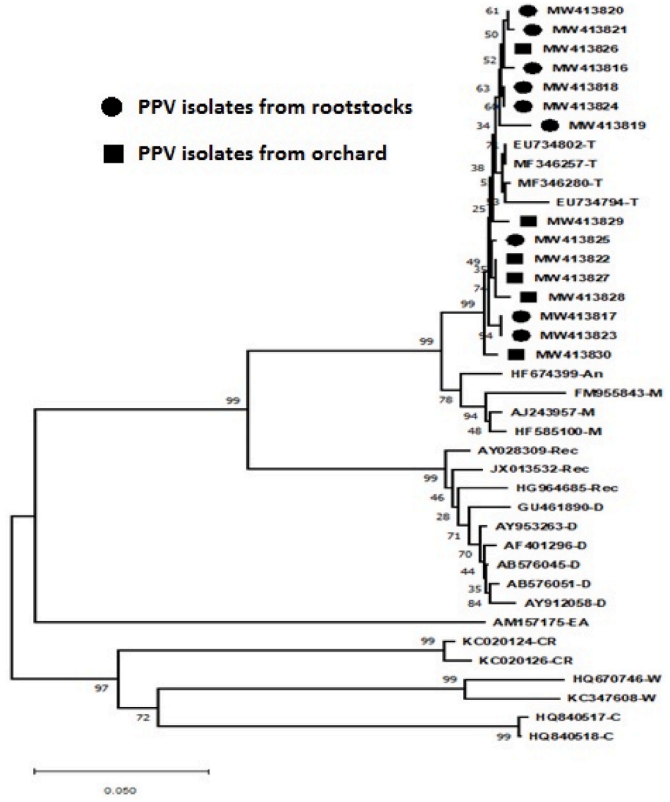


Fig. 2. Phylogenetic analysis of PPV-T isolates using neighbor-joining method with 1000 bootstrap value. Samples studied in the current experiment were shown as bold square and circle. The rest of the sequences were downloaded from NCBI database.

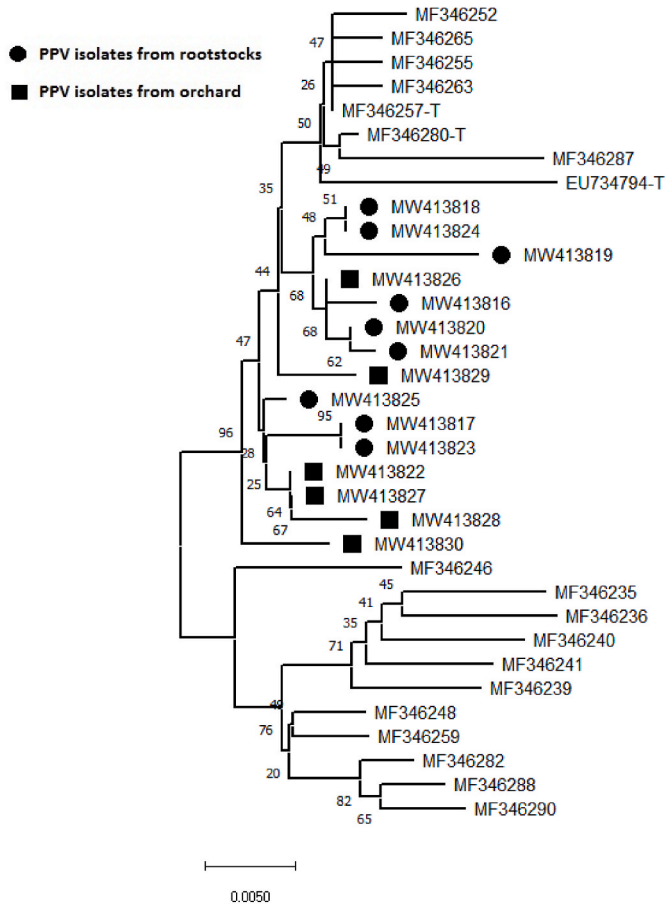


Fig. 3. Phylogenetic analysis of PPV-T isolates using neighbor-joining method with 1000 bootstrap value. Samples studied in the current experiment were shown as bold square and circle. The rest of the sequences were downloaded from NCBI database.

however, it started to rise from the beginning of June to the first half of July. After the second week of July, the aphid population decreased (Fig. 4).

In 2015, the highest percentage of viruliferous aphids was found in *A. gossypii* (100%), followed by *B. helichrysi* (77.77%), *A. spiraecola* (55.72%), *M. persicae* (46.05%), *H. pruni* (30.76%) and unidentified aphid species (30.76%) whereas it was 100% for *A. gossypii* and *M. euphorbiae* followed by *A. spiraecola* (76.32%), *M. persicae* (73.1%), *B. helichrysi* (61.53%), unidentified aphid species (50%), and *H. pruni* (62.06%) in 2016 (Table 2). During 2015–2016 the most frequent aphid species that are known as PPV vectors, captured in the in the experimental orchard belonged to the genus Aphis (*A. gossypii* and *A. spiraecola*).

During the two years experiments, the highest percentage of viruliferous aphids were mostly found on Myrobalan 29B, Garnem, Myrobalan 29C, GF 677, Nemaguard. The two most common aphid species, *A. gossypii* and *A. spiraecola* were viruliferous at the highest percentage (Tables 3 and 4).

4. Discussion

The PPV isolates found in the *Prunus* orchard (inoculum source) and in the rootstocks of the experimental plot were found as PPV-T strain, which is a common strain in stone fruit plantations in Kayseri province, located in Central Anatolia region of Turkey. The PPV-T strain was already found to be widespread in residential gardens in Ankara, Izmir, Istanbul, Kayseri, Konya, and Thrace (Edirne, Kırklareli, and Tekirdag provinces) [7,34]. The presence of PPV-T is not suprising since this strain is the most common one in Central Anatolia and most of the regions in Turkey [8,23]. although all PPV-T and PPV-M strains clustered together which consistent with the previous studies, some PPV-M

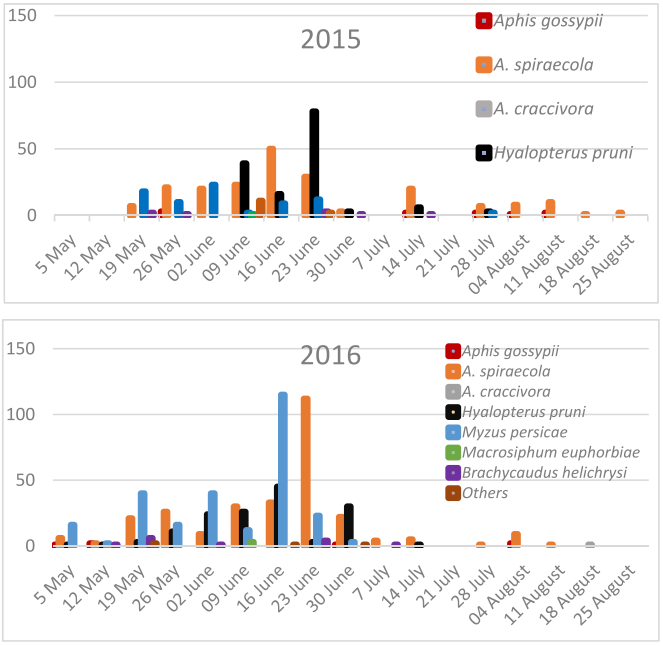


Fig. 4. Mean number of aphid species caught each week using the sticky-plant method in the experimental plot established with five different *Prunus* rootstocks.

Table 2
Occurrence of PPV-vector aphid species in the experimental plot in Kayseri. Number of individuals caught and their percentage among total aphid numbers in the seasons of 2015 and 2016 (May–August).

Aphid species	2015	Percentage %	2016	Percentage %
	Number of identified aphid		Number of identified aphid	
Myzus persicae	76	16.85	264	36.71
Aphis gossypii	10	2.21	6	0.83
A. spiraecola	201	44.56	283	39.36
Hyalopterus pruni	143	31.70	145	20.16
Brachycaudus helichrysi	9	1.99	13	1.80
A. craccivora	0	0	1	0.13
Macrosiphum euphorbiae	1	0.22	3	0.41
Others	11	2.43	4	0.55
Total	451		719	

samples from Adriatic basin separated from the Anatolian samples [8,23].

In this study, under severe PPV inoculum pressure, the studied rootstocks exhibited differences in their susceptibility to PPV-T infection by aphids (Table 1). Nemaguard, Myrobalan 29B and Myrobalan 29C proved highly susceptible, with infection rates of 3.61%, 2.74% and 1.04% in both years respectively, in agreement with results from similar studies (Vidal et al., 2010 [35]; Caglayan et al., 2013; Kaya et al., 2014). Although less percentage viruliferous aphids were detected on Nemaguard, it was found the highest infected rootstock for both 2015 and 2016. This situation can be explained by the highest number of viruliferous aphids visiting this rootstock for both years (Tables 3 and 4). Nemaguard was also reported as one of the most susceptible rootstocks to natural PPV infection in Spain [12], Czech Republic [36] and Bulgaria [35]. The susceptibility observed in Nemaguard, an interspecific cross between peach and *P. davidiana*, agrees with the results reported by Refs. [37,38]; indicating a certain level of susceptibility to a PPV-M and PPV-D isolates, respectively. Although it is known that *P. cerasifera* is susceptible to PPV [39], different clones have been reported as resistant to PPV-D [10,11]. These results confirm the high susceptibility of Myrobalan 29C, Myrobalan 29B and Nemaguard to PPV-T but the symptoms on Myrobalan 29B and 29C were always much milder than Nemaguard. GF 677 and Garnem was described as tolerant to PPV-T isolates in the current study confirming previous studies reported in our country by Refs. [21,22]. The characterization of the resistance/-susceptibility level of the rootstock is of priority in the search for new PPV resistance sources within *Prunus* spp. and the results presented here indicate high susceptibility to PPV within the genus.

This is the first study on epidemiological aspect of PPV-T in Central Anatolia region of Turkey where PPV is widespread. PPV has been transmitted by at least 20 aphid species, although only 4–6 are considered important vectors and many of them are likely to occur in Turkey. The most numerous migrant aphids trapped in Kayseri was *A. spiraecola* and it was already reported as an efficient vector of PPV in the Mediterranean region of Turkey [22]. The second and third most numerous species trapped in the experimental plot were *M. persicae* and *H. pruni*. *Myzus persicae* populations are widely used for transmission tests, and it is one of the most efficient PPV vector in France and Spain [40,41].

Variation in the number of captured aphids in plum and apricot orchards may be explained by many factors that influence population dynamics of aphids. The major potential factors are, available plant hosts, predators, pathogens, plant quality, intensity of pesticide application and weather conditions (temperature and precipitation) [42]. In the present study, significant number of aphid species were captured in June (Fig. 3). Inconsistently, similar studies those were performed in the West [21] and South [22] parts of Turkey showed that the highest aphid population was observed earlier which was in the last week of May. When these findings are compared with the results of the current study, wehereas South and West Turkey show typical Mediterranean climate and close to sea level, Central Anatolia shows harsh and arid climatic conditions and approximately 1000 m above from the sea level. Similar to Turkey's Mediterranean climate zones, the seasonal dynamics of captured aphid species on plum during three investigated years was presented in Serbia by Ref. [31]. There, they also found that highest aphid populations were observed in May and June [31]. Using yellow sticky traps, the autumn peaks also were confirmed in apricot tree orchards in Austria and Hungary, but this did not occur in the South-East of France [43]. Using the sticky shoot method, the second peak in autumn was reported in Romania and in Bulgaria, but not in Spain [12,43,44]. [43] explained that this autumn peak of flight activity is more

Table 3
The number of viruliferous aphids/total number of aphid species collected from different *Prunus* rootstocks in the experimental plot in 2015.

Aphid species	Nemaguard	Myrobalan 29B	Myrobalan 29C	GF 677	Garnem	Total
Aphis gossypii	0/0	1/1	2/2	0/0	7/7	10/10 (100%)
A. spiraecola	26/44	18/28	22/43	23/40	23/46	112/201 (55.72%)
A. craccivora	0/0	0/0	0/0	0/0	0/0	0/0
Hyalopterus pruni	18/92	8/12	3/13	12/21	3/5	44/143 (30.76%)
Myzus persicae	15/44	12/12	7/8	1/12	0/0	35/76 (46.05%)
Macrosiphum euphorbiae	0/0	0/0	0/1	0/0	0/0	0/1
Brachycaudus helichrysi	3/4	1/1	0/0	3/4	0/0	7/9 (77.77%)
Others	4/11	0/2	0/0	0/0	0/0	4/13 (30.76%)
Total	66/195 (33.85%)	40/56 (71.42%)	34/67 (50.74%)	39/77 (50.64%)	33/58 (56.89%)	212/453 (46.79%)

Table 4
The number of viruliferous aphids/total number of aphid species collected from different *Prunus* rootstocks in the experimental plot in 2016.

Aphid species	Nemaguard	Myrobalan 29B	Myrobalan 29C	GF 677	Garnem	Total
Aphis gossypii	1/1	2/2	0/0	3/3	0/0	6/6 (100%)
A. spiraecola	64/70	21/31	34/61	43/57	54/64	216/283 (76.32%)
A. craccivora	0/0	0/0	0/1	0/0	0/0	0/1
Hyalopterus pruni	50/74	3/5	21/34	12/16	4/16	90/145 (62.06%)
Myzus persicae	78/130	4/4	10/12	51/60	50/58	193/264 (73.1%)
Macrosiphum euphorbiae	0/0	3/3	0/0	0/0	0/0	3/3 (100%)
Brachycaudus helichrysi	0/0	0/0	4/8	0/0	4/5	8/13 (61.53%)
Others	0/0	1/1	1/1	0/2	0/0	2/4 (50%)
Total	193/275 (70.18%)	34/46 (73.91%)	70/117 (59.82%)	109/138 (78.98%)	112/143 (78.32%)	518/719 (72.04%)

important in the regions with cold winters where aphid populations exclusively overwinter as eggs.

In 2015, *A. spiraecola* and *H. pruni* were dominant whereas, in 2016, *A. spiraecola* and *M. persicae* were more dominant than other aphid species (Table 2). For epidemiological purposes, it is important to take into consideration the aphid species as well as the number of viruliferous aphids visiting the nursery plots. The most important PPV vectors reported from several countries are *B. cardui, B. helichrysi, M. persicae* and *Phorodon humuli* [45] and *A. spiraecola* [2]. Natural virus spread is low in summer but high in spring and autumn. Spring flights of *B. helichrysi, M. persicae*, and *P. humuli* are the most important for virus spread within and between orchards [45]. In south of Turkey where close to Syria border, the dominant aphid species were *A. spiraecola* and *A. gossypii*, showing a peak in May in both 2011 and 2012; and the percent ages of PPV-viruliferous aphids were 12.5% in *A. spiraecola*, 12.42% in *A. gossypii*, and 11.11% in *H. pruni* [22]. Based on the fact that the virus-positive insect rate for both *A. spiraecola* and *A. gossypii* was high, they are likely to pose a high PPV transmission risk [33]. Therefore, aphids may carry the virus in their intestinal tract after having fed on a PPV-infected tree without being a vector of PPV [46]. However, the detection of PPV in flying aphids is a very important indication that active sources of PPV are present in or around the area, meaning a high risk of spreading PPV disease in cases where PPV-positive aphids, particularly *A. spiraecola* and *A. gossypii*, were detected in the area. For epidemiological purposes, it is important to take into consideration the aphid vector species as well as the number of PPV-viruliferous aphids visiting the nursery plots. Air temperature has previously been shown to be important in determining the detectability of virus in sticky-trapped thrips [47]. Atmospheric temperatures vary between seasons and sites according to region, aspect, elevation, and exposure. This is highlighted by extensive recognition of temperature as a factor in the interannual variability of aphid population dynamics contributing to virus epidemiology [13,48,49].

In conclusion, the combined use of resistant or less susceptible PPV rootstocks, such as Garnem and GF 677, together with environmentally friendly treatments with zero residues could constitute an integrated strategy to reduce PPV spread in the nursery industry. Combined with accurate detection methods, our findings will play an important role in PPV control in nurseries, which are the most frequent sources of long-distance distributions of infected material worldwide. These strategies could be also applied to prevent/reduce the PPV spread in new orchards. The scarcity of resistant rootstocks for the different stone fruits highlights the need for breeding PPV resistant rootstocks for these species. Feeding this data into an aggregated model would help to reliably forecast the economic risk posed to an individual crop following primary infection and secondary spread of the disease, taking into account growth stage at vector ingression and seasonal temperatures. Understanding of the complex nature of PPV epidemiology, including the influence of surrounding land use, could be improved using this tool and contribute to the establishment of economic thresholds for insecticide application.

CRediT authorship contribution statement

Cemile Temur Cinar: Data curation, Formal analysis, Writing – review & editing. **Mona Gazel:** Data curation, Writing – review & editing. **Kamuran Kaya:** Data curation, Writing – review & editing. **Antonio Olmos:** Review & editing, Supervision. **Kadriye Caglayan:** Conceptualization, Methodology, Funding acquisition, Resources, Project administration, Writing – review & editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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