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Sharka, an Important Quarantine Disease for California: Risk Assessment through Almond Cultivars and Rootstocks Phenotyping

Manuel Rubio, Federico Dicenta, Pedro J. Martínez-García, and Pedro Martínez-Gómez
Department of Plant Breeding, CEBAS-CSIC, PO Box 164, 30100 Espinardo, Murcia, Spain

Theodore Dejong and Thomas Gradziel
Department of Plant Sciences, University of California Davis, Davis, CA, USA

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Abstract. **Sharka is among the most important diseases of stone fruits globally. It is caused by the *Potyvirus plumpoxi* [plum pox virus (PPV)] and affects mainly apricot, plum, prune, and peach trees. In 1999, PPV was detected for the first time in the United States, and it was declared eradicated by the US Department of Agriculture in Oct 2019. Despite the official declaration of eradication, Sharka is still of great concern, mainly because of the huge quarantine efforts and millions of dollars spent on eradicating it. Studies of vulnerability of Californian almond cultivars and rootstocks to Sharka are scarce, and the reactions of different cultivars and rootstocks to Sharka were never thoroughly analyzed. The main objective of this study was to assess the risk of the potential damage of PPV to almond orchards in California and the subsequent dispersion of the virus to other *Prunus* tree crops by evaluating the susceptibility and tolerance of the most cultivated almond cultivars and rootstocks. A group of cultivars and rootstocks were evaluated to determine their vulnerability to PPV-Dideron. After 4 years of analyses with more than 1100 tests performed, a total of 11 almond cultivars and 17 rootstocks were infected. None of the almond cultivar replicates that tested positive (21) displayed Sharka symptoms. However, most of the 19 rootstocks assays displayed significant Sharka symptoms and tested positive (327). These results indicate the importance of rootstocks in a potential Sharka disease outbreak in California. In addition, almond tree infection was detected for the first time with our isolate, but only in a small number of trees.**

Stone fruits (*Prunus sp.*) are affected by many viral diseases that can cause important economic losses (Rubio et al. 2017). Sharka disease, caused by Plum pox virus (PPV), is the most damaging of these viruses and induces extensive losses of Japanese plum (*P. salicina* L.), prune (*P. domestica* L.), apricot (*P. armeniaca* L.), sweet cherry (*P. avium* L.), sour cherry (*P. cerasus* L.) and peach [*P. persica* (L.) Batsch] through the reduction of fruit quality, premature fruit drop, and rapid natural virus spread by aphid vectors (Sihelská et al. 2017). This agent has been classified as a quarantine pathogen and as one of the top 10 viruses in crops (Scholthof et al. 2011); additionally, it has recently been described among the most important global pandemic diseases (Jones 2021).

Since its first description in Bulgaria in 1917 (Atanassov 1932), Sharka has spread to most of the temperate fruit production areas of the world (Rubio et al. 2019). Presently, only Oceania and South Africa have not reported any incidence of the virus (García et al. 2025). In the United States, PPV was detected in 1999 in Pennsylvania (Levy et al. 2000) and was declared eradicated by the US Department of Agriculture in Oct 2019 (https://www.aphis.usda.gov/aphis/newsroom/news/sa_by_date/sa-2019/plum-pox-declaration) after 20 years of eradication efforts. The global cost of Sharka damage could exceed €13 billion (≈$14 billion) over the last century (Cambra et al. 2024). This high figure includes direct costs, such as yield losses as well as eradication and disease removal programs, border rejections, and compensatory measures, and indirect costs related to prevention, diagnostics, trade restrictions, and research (Cambra et al. 2024). In the United States, the cost directly linked with early eradication programs was more than €30.3 million, with an indirect cost of approximately €90 million, through investment in research projects of different organizations (US Department of Agriculture, Agricultural Research Service, Animal and Plant Health Inspection Service) and funding sources to eliminate PPV (Welliver et al. 2014).

Although PPV is characterized by its wide genetic variability, the following 10 independent strains that vary in sequence, localization, and potential hosts have been described: Dideron (D), Marcus (M), El Amar (EA), Recombinant D and M (Rec), Turkey (T), Cherry (C), Cherry Russian (CR), Cherry Volga (CV), Winona (W) and Ancestor (An) (García et al. 2025). However, D is the prevalent strain of PPV; it is widespread globally and present in practically every country in which PPV has been detected (García et al. 2014). These multiple types demonstrate the dangerous capacity of PPV to mutate and change. Additionally, aphids transmit the virus in a nonpersistent way. Aphids that have been feeding on infected plants can transmit for up to 3 h after acquisition (Kunze and Krozal 1971). Many aphid species have been reported as vectors, including *Aphis gossypii*, *A. craccivora*, *A. fabae*, *A. spiraecola*, *Brauchycaudus helichrysi*, *B. cardui*, *Myzus persicae*, *M. varians*, *Hyalopterus pruni*, and *Phorodon humuli* (Kunze and Krozal 1971; Llácer et al. 1992). Additionally, PPV can be spread in orchards by transient aphids as efficiently as colonization of *Prunus* by aphids (Gottwald et al. 1995). However, long-distance dissemination usually occurs by vegetative propagation and usage of infected cultivars and rootstocks. Seed transmission has been reported once (Németh and Kölber 1982), but the most accepted hypothesis now is that PPV is not seed-transmitted (Eynard et al. 1991; Triolo et al. 1993).

Despite the official declaration of eradication in the US, Sharka is still of great importance in California, even though it has never been described there. Furthermore, PPV was detected in Mexico (Loera-Muro et al. 2017), which is much closer to California than the counties where PPV was detected in the United States. Therefore, authorities, nurseries, and farmers (stakeholders) must stay alert to this potential threat.

The almond acreage in California is more than 0.65 million ha (1.63 million acres). In 2023, it reached more than 1 million tons (2.34 billion pounds) of almond kernel production. Therefore, even a rare Sharka infection could result in the rapid multiplication of PPV in almond orchards, and the infected trees could act as a virus source for other fruits cultivated nearby.

Fortunately, Sharka disease in almonds has been rarely reported. Many years ago, almond was described as a non-PPV host (Kölber 2001; Németh 1994), and some

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M.R. is the corresponding author. E-mail: mrubio@cebas.csic.es.

almond cultivars were described as resistant (Pascal et al. 2002; Rubio et al. 2003). Twenty years ago, our results showed a high level of resistance to the type D strain by the almond cultivars and that this resistance could be transmitted successfully to the interspecific almond × peach progenies (Martínez-Gómez et al. 2004). However, after mechanical inoculation, other researchers successfully infected young almond seedlings (Festic 1978). Pribék et al. (2001) described the presence of a type D isolate that infected almond plants. Dallot et al. (1997) also experimentally infected the Aï almond cultivar by grafting and via aphid transmission from a diseased 'GF305' peach. Recently, Rogers et al. (2024) demonstrated that Tuono and Mission cultivars can be infected by the US isolate PPV-D (Penn4), and that 'Tuono' is a transmission-competent host. Both teams were able to infect healthy GF305 using aphids that had fed on diseased almonds.

Rootstocks from nurseries can also play an important role in disease spread because of their clonal propagation, high planting density, and the fact that the juvenile stage of plants is very susceptible to infection. In the case of PPV, nursery conditions are particularly critical because young plants in the nursery are especially susceptible to aphids, the natural vector of this disease. Once in the field, direct infection of rootstocks is unlikely, except for those with a tendency to produce suckers, which are frequently visited and colonized by aphids and, therefore, likely to be infected with PPV (Rubio et al. 2019). Consequently, the uncertain behavior of almond trees against PPV and the threat posed to overall stone fruit production in California need to be addressed.

Management of Sharka disease is not an easy task, as has been demonstrated by its rapid global distribution to many production areas. Control of Sharka needs to be based on knowledge about the epidemiology, which, in turn, depends on multiple parameters inherent in virus isolates, host plants, vector populations, culture practices, and environmental conditions (Rimbaud et al. 2015). Recently, different epidemiological mathematical models have been developed to help predict Sharka outbreaks and subsequent viral spread and optimize the measures for disease control (Gutiérrez-Jara et al. 2023; Rimbaud et al. 2019; Vidal et al. 2020).

The objective of this study was to assess the risk of PPV development in the almond orchards in California including its subsequent dispersion to other stone fruit orchards by evaluating the susceptibility and tolerance of the most cultivated almond cultivars and rootstocks.

Materials and Methods

Plant material

We performed three different assays, two by top-grafting almond cultivars and some rootstocks (tested as scions) onto Sharka-inoculated 'GF305' peach seedlings, used as a biological indicator (Bernhard et al. 1969), and one as a set of own-rooted rootstocks directly inoculated with 'GF305'-infected buds. Tables 1 and 2 describe the main characteristics of 23 almond cultivars and 19 rootstocks studied.

The number of replicates tested for each cultivar and rootstock varied. Initially, we focused on the main cultivars/rootstocks for the almond industry in California. However, the European Union has limited the importation of plant material from countries with *Xylella fastidiosa*. Therefore, we looked for American genotypes that already had been introduced into the European Union and could be of significance to the California almond industry. Some of the cultivars with very low replicates are linked with very old almond trees conserved in Europe collections and were included to increase the genetic variability tested. Regarding the grafted rootstocks, there was only one way to test them because it was impossible to import seeds or scions from the United States and they were not available at the commercial level in our local nurseries.

The PPV isolate

We performed the PPV inoculations with the PPV-D isolate 3.30RB/GF-IVIA (GenBank: KJ849228.1), which is considered a representative isolate of the Spanish population, originally collected on *Prunus salicina* (Red Beaut) and later maintained on GF305 peach seedlings in our facilities at CEBAS-CSIC in Murcia (Spain).

Phenotyping procedure

The phenotyping process of PPV is a protracted procedure that includes several steps to thoroughly determine the behavior of each cultivar or rootstock against the virus. This approach encompasses three phenotyping cycles that require at least 2 years of study. In the present work, we followed two independent methodologies depending on the nature of the cultivar or rootstock tested (Fig. 1).

First, we obtained the rootstock seedlings from 'GF305' peach by stratification for 16 weeks at 7 °C. Later, germinated seeds were sown in 3.5 L pots (0.93 gallons). After 8 to 10 weeks of growth, seedlings were inoculated by grafting with a piece of bark of infected GF305 seedlings showing severe Sharka symptoms.

Evaluation of cultivars onto infected GF305. During this assay, we evaluated PPV-D against 23 almond cultivars [Alaska, All in One, Butte, Carmel, Florida, Fritz, Independence, Makako, Marcona, Mono, Monterey, Mission (Texas), Ne Plus Ultra, Nonpareil, Padre, Peerless, Penta, Price Cluster, Sonora, Tardy Nonpareil, Tioga, Wawona, and Wood Colony]. At 1 month after inoculation of GF305, at least five replicates of

Table 1. Almond cultivars assayed.

Almond cultivar	Pedigree	Origin	Floral compatibility	Blooming time	Ripening time
Alaska	D00-078 × D02-462	Spain	Self-compatible	Extra-late	Intermediate
All in one	*P. dulcis* × *P. persica*	USA	Self-compatible	Late	Late
Butte	Unknown	USA	Self-incompatible	Late	Intermediate
Carmel	Mutation of Nonpareil	USA	Self-incompatible	Late	Late
Florida	Antoñeta × Marcona	Spain	Self-compatible	Early	Extra-early
Fritz	Mission × Drake	USA	Self-incompatible	Intermediate	Intermediate
Independence	*P. dulcis* × *P. persica*	USA	Self-compatible	Late	Late
Makako	Lauranne × S5133	Spain	Self-compatible	Extra-late	Late
Marcona	Unknown	Spain	Self-incompatible	Early	Early
Mission (Texas)	Unknown	USA	Self-incompatible	Intermediate	Intermediate
Mono	Unknown	USA	Self-incompatible	Late	Late
Monterey	Unknown	USA	Self-incompatible	Late	Late
Ne plus ultra	Unknown	USA	Self-incompatible	Early	Late
Nonpareil	Unknown	USA	Self-incompatible	Late	Late
Padre	Mission × Swanson	USA	Self-incompatible	Late	Late
Peerless	Unknown	USA	Self-incompatible	Intermediate	Intermediate
Penta	S5133 × Lauranne	Spain	Self-compatible	Extra-late	Late
Price Cluster	Nonpareil × Mission	USA	Self-incompatible	Late	Late
Sonora	Nonpareil × Eureka (BC)	USA	Self-incompatible	Intermediate	Intermediate
Tardy Nonpareil	Mutation of Nonpareil	USA	Self-incompatible	Late	Late
Tioga	Unknown	USA	Self-incompatible	Late	Intermediate
Wawona	Rubi × Mission	USA	Self-incompatible	Late	Late
Wood Colony	Unknown	USA	Self-incompatible	Late	Late

Table 2. Almond rootstocks assayed.

Rootstock	Species	Origin	Propagation	Compatibility	Vigor	Suckers
Adesoto101 (Empyrean 101)	*Prunus insititia*	Spain	In vitro	Apricot, peach, plum, prune	Low	High
Cadaman	*P. persica* × *P. davidiana*	France	In vitro	Almond, peach	Medium	Low
Citation	*P. salicina* × *P. persica*	USA	Cuttings	Apricot, plum	Low	Low
DryStock™ One	*Prunus dulcis*	Spain	Seeds	Almond	High	Low
Garnem	*P. dulcis* × *P. persica*	Spain	In vitro	Almond, peach	High	High
GF305	*Prunus persica*	France	Seeds	Almond, peach, apricot	Medium	Low-medium
GF677 (Paramount®)	*P. dulcis* × *P. persica*	France	In vitro	Almond, peach	High	Low
Guardian	*Prunus persica*	USA	Seeds	Peach	Medium	Low
Hansen 536	*P. dulcis* × *P. persica*	USA	Cuttings	Almond, peach, plum	High	Low
Krymsk 86 (Kuban 86)	*P. cerasifera* × *P. persica*	Russia	Cuttings	Peach, plum, prune	High	Some
Lovell	*Prunus persica*	USA	Seeds	Almond, apricot, peach, plum	Medium	Low
Marianna 2624	*P. munsoniana* × *P. cerasifera*	USA	Cuttings	Apricot, plum	Medium	High
Nemaguard	*P. persica* × *P. davidiana*	USA	Seeds	Almond, peach	Medium	Low
Nemared	Sport of Nemaguard	USA	Seeds	Almond, peach	Medium	Low
Penta (Empyrean 2)	*Prunus domestica*	Italy	Seeds	Almond, apricot, peach, plum	Low	Medium
Rootpac 20	*P. besseyi* × *P. cerasifera*	Spain	In vitro	Almond, peach, plume	Low	High
Rootpac R (Mirobac)	*P. cerasifera* × *P. dulcis*	Spain	In vitro	Almond, peach, plume	Medium	Medium
Tetra (Empyrean 3)	*Prunus domestica*	Italy	Seeds	Almond, apricot, peach, plum	Low	Medium
Viking	Complex *Prunus* hybrid	USA	Cuttings	Almond, peach	Medium	Low

each selected scion were grafted onto the inoculated GF305 peach seedling.

Evaluation of rootstocks onto infected GF305. In this trial, we evaluated PPV-D against nine commercial rootstocks ['Citation', 'Guardian', 'Hansen 536', 'Lovell', 'Nemaguard', 'Nemared', 'Penta' (Empyrean 2), 'Tetra' (Empyrean 3) and 'Viking™'] using the same procedure as that used for the almond cultivars.

Evaluation of rootstocks growing onto their own roots. During this assay, seedling rootstocks ('DryStock™ One' and 'GF305') were stratified at 7 °C, and vegetatively multiplied rootstocks ('Adesoto 101', 'Cadaman', 'Garnem', 'GF677', 'Krymsk® 86', 'Marianna 2624', 'Rootpac®R' and 'Rootpac® 20') were acquired in local nurseries that supplied them in small 150 mL (5 fl oz) pots; subsequently, they were transferred to 3.5 L pots. The inoculation was performed directly on the own-rooted rootstocks by grafting a bud from an infected GF305 peach seedling. Fifteen replicates of each rootstock were initially included in the phenotyping process.

To accelerate viral multiplication and symptom expression, plants were submitted to artificial growth cycles with rest periods in a cool chamber (artificial winters: December–January and July–August) and growing periods in a greenhouse (artificial springs: February–June and September–November). During the winter cycle, plants were placed at 7 °C and in darkness for 2 months and then moved to a greenhouse under controlled conditions (Fig. 1).

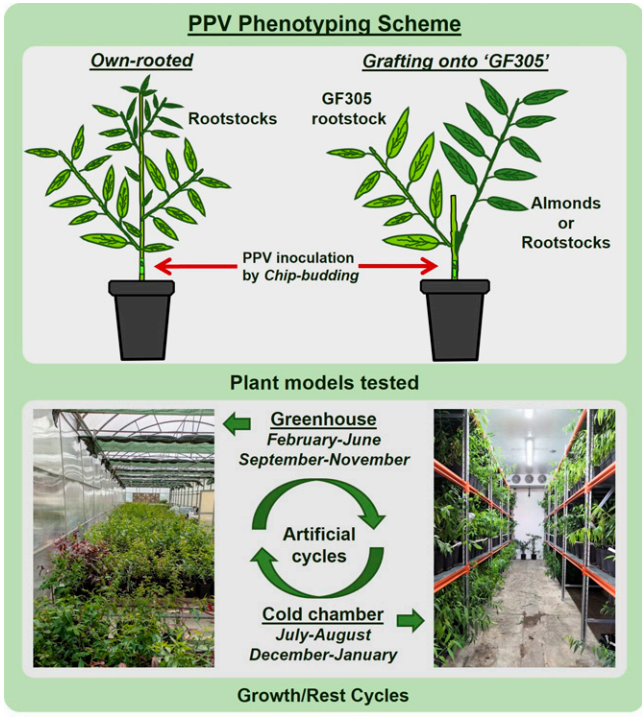

Fig. 1. Plum pox virus (PPV) phenotyping process. Plant models tested: own-rooted rootstock scheme (left) and 'GF305' rootstock infected with PPV and grafted with the genotypes to be tested (right). Pictures show the greenhouse where the evaluation has been performed and the cold chamber where the plants had been submitted to artificial rest periods.

After 8 to 10 weeks of growth in the greenhouse, when vegetative buds sprouted, we performed the PPV evaluation. Sharka symptoms were scored on leaves of rootstock and scions using a scale from 0 to 5 by considering intensity and distribution in the plant as follows: 0, no symptoms; 1, discrete chlorosis or spots restricted to one or two leaves; 2, slight chlorosis bordering leaf veins on three or more leaves; 3, vein chlorosis or rings on numerous leaves; 4, chlorosis, rings and some distortions on most leaves; and 5, strong chlorosis or distortions on all leaves.

Reverse-transcription polymerase chain reaction procedure

The presence of PPV was confirmed by a conventional reverse-transcription (RT) polymerase chain reaction (PCR) analysis. Total RNA was extracted from leaves using an adapted CTAB method for plants described by Tong et al. (2012). Two specific primers within the CP gene, VP337 (CTCTGTGTCCTCTTCTTGTG), complementary to positions 9487–9508, and VP338 (CAATAAAGCCATTGTTGGATC), complementary to positions 9194–9216, were used (Sánchez-Navarro et al. 2005). The enzymes used were Avian Myeloblastosis virus RT (AMV RT) and GoTaq®Flexi DNA polymerase (Promega, Madison, WI, USA). The RT-PCR was performed using a SimpliAmp™ thermal cycler (Applied Biosystems, Thermo Fisher, Waltham, MA, USA), and the parameters were as follows: one cycle of 42 °C during 54 min (cDNA synthesis) followed by a cycle at 94 °C for 2 min and 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension cycle of 72 °C for 5 min. The RT-PCR amplified products were electrophoresed in 1.5% agarose gels in 40 mM Tris-acetate and 1 mM EDTA (pH 8.0) and stained with Gel Red® (Biotium, Freemont, CA, USA); the expected amplicon size was 313 bp.

Table 3. Summary of the behavior against plum pox virus Dideron (PPV-D) isolate 3.30RB/GF-IVIA after three evaluation cycles of the three assays performed: grafted almonds, grafted rootstocks and own-rooted rootstocks.

		Cycle 1			Cycle 2			Cycle 3			Global			
		GF305	Cultivar		GF305	Cultivar		GF305	Cultivar		GF305	Cultivar		
Assay	N	Symp.	Symp.	RT+/ Total	Symp.	Symp.	RT+/ Total	Symp.	Symp.	RT+/ Total	Symp.	Symp.	RT+/ Total	% Positives
Grafted almond	198	195 (3.0)	0 (0.0)	9/195	160 (2.6)	0 (0.0)	6/160	127 (1.6)	0 (0.0)	6/143	482 (2.5)	0 (0.0)	21/498	4.2%
Grafted rootstock	66	64 (3.9)	55 (2.0)	59/64	54 (2.1)	13 (2.1)	21/54	51(2.6)	12 (2.1)	16/54	169 (2.9)	80 (2.0)	96/172	55.8%
Own-rooted Rootstock	151	—	77 (2.9)	77/151	—	73 (2.1)	77/151	—	67 (2.2)	69/141	—	217 (2.6)	223/443	50.3%
Total	415	259 (3.2)	132 (2.5)	145/410	214 (2.5)	86 (2.1)	104/365	178 (1.9)	79 (2.2)	91/338	651 (2.6)	297 (2.3)	340/1113	30.5%

% Positives = percentage of positive reverse-transcription (RT) polymerase chain reaction (PCR) results after the three phenotyping cycles; N = number of plants tested; RT+/Total = number of positive RT-PCR results over the total number of plants correctly phenotyped; Symp. = number of infected plants (mean symptom intensity scored from 0 to 5).

The greenhouse evaluation process was performed for 3 growing cycles. All plants were reinoculated when they did not display Sharka symptoms or the RT-PCR was negative after the first and the second cycles of phenotyping.

Results

A total of 415 plants (198, 66, and 151, respectively) included in the three assays (almond cultivars and rootstocks grafted on 'GF305' and rootstocks evaluated on their own roots) were studied (Table 3). The final number of phenotypic observations recorded after three cycles was 1113 (410 + 365+338); of these, 340 had positive RT-PCR results. Almond cultivars were less susceptible than rootstocks, with 4.2% yielding positive RT-PCR results. In contrast, rootstocks grafted and grown on their own roots reached 55.8% and 50.3% respectively. A remarkably high inoculation success rate of almost 98% (195/198 + 64/66) for infected GF305 was observed. The average symptom intensity on the GF305 rootstocks decreased from 3.2 in the first cycle to 1.9 in the third cycle. Approximately 25% of the replicates of almond tree cultivars were lost between the beginning and the end of the experiment.

Evaluation of almond cultivars

A total of 198 plants of 23 almond cultivars were studied; the number of replicates per cultivar differed because of the starting plant material, inoculation, and grafting success. A decrease in the symptomatology on the GF305 rootstock was observed throughout the three cycles (from 3.0 in the first cycle to 1.6 in the third), although levels were sufficient to ensure inoculum pressure on the cultivar. None of the cultivars studied showed Sharka symptoms throughout the study (Fig. 2). However, we were able to detect for the first time the presence of the PPV-D isolate 3.30 RB/GF-IVIA on almond leaves, thus confirming 21 RT-PCR positive results among the 498 performed (4.2%) (Table 4).

Padre and Fritz showed the highest percentage of positive RT-PCR results, but the levels were always low. Additionally, PPV was detected on Mission, Nonpareil, Butte, Mono, AK, Price Cluster, Makako, Wood Colony, and Florida. Regarding the other 12 cultivars tested, PPV was never detected despite having been studied for three growing cycles, and the GF305 rootstock was verified as infected and showed Sharka symptoms. There was no relation between symptom intensity on GF305 and the RT-PCR positive or negative results.

Fig. 2. Sharka test for almond cultivars. In the photos, Sharka symptoms are seen on the GF305 peach rootstocks. The almond cultivars lack symptoms. However, some almond cultivars—such as Padre, Fritz, Mission, Nonpareil, Butte, AK, Price Cluster, Wood Colony and Florida —had positive reverse-transcription polymerase chain reaction results. This implies that PPV-D particles could infect the almond tissue without resulting in visible Sharka symptoms. Other cultivars, such as Independence, Carmel, and Monterey, have been fully resistant.

Table 4. Evaluation of the behavior of almond cultivars grafted onto infected 'GF305' against a plum pox virus Dideron (PPV-D) isolate 3.30RB/GF-IVIA sorted by susceptibility.

		Cycle 1		Cycle 2		Cycle 3		Global		
		GF305	Almond	GF305	Almond	GF305	Almond	GF305	Almond	
Almond cultivar	N	Symptoms	RT+/Total	Symptoms	RT+/Total	Symptoms	RT+/Total	Symptoms	RT+/Total	% Positives
Padre	11	11 (2.9)	2/11	10 (3.1)	0/10	8 (1.2)	1/8	29 (2.5)	3/29	10.3
Fritz	11	11 (3.6)	1/11	9 (2.7)	2/9	9 (2.4)	0/10	29 (2.9)	3/30	10.0
Mission (Texas)	10	9 (2.6)	1/9	10 (2.0)	1/10	5 (2.2)	0/6	24 (2.3)	2/25	8.0
Nonpareil	17	17 (3.0)	2/17	14 (2.6)	1/14	8 (1.9)	0/8	39 (2.6)	3/39	7.7
Butte	13	13 (3.5)	2/13	8 (2.3)	0/8	5 (1.6)	0/6	26 (2.8)	2/27	7.4
Mono	6	6 (2.2)	0/6	4 (2.0)	0/4	2 (1.0)	1/4	12 (1.9)	1/14	7.1
Alaska	6	6 (2.7)	0/6	4 (2.8)	0/4	4 (1.2)	1/5	14 (2.3)	1/15	6.7
Price Cluster	7	7 (2.0)	0/7	5 (1.5)	0/5	4 (1.5)	1/5	16 (1.7)	1/17	5.9
Makako	16	16 (2.6)	1/16	13 (2.4)	0/13	11 (1.4)	1/15	40 (2.2)	2/44	4.5
Wood Colony	9	9 (3.8)	0/9	8 (2.4)	1/8	5 (1.6)	0/5	22 (2.8)	1/22	4.5
Florida	16	16 (3.1)	0/16	14 (2.7)	1/14	13 (1.5)	1/14	43 (2.5)	2/44	4.5
Independence	19	19 (3.2)	0/19	19 (3.1)	0/19	19 (1.5)	0/19	57 (2.6)	0/57	0.0
Carmel	13	11 (3.6)	0/11	12 (2.6)	0/12	8 (1.9)	0/8	31 (2.8)	0/31	0.0
Monterey	11	11 (3.8)	0/11	8 (2.9)	0/8	7 (1.3)	0/7	26 (2.8)	0/26	0.0
Penta CEBAS	8	8 (1.7)	0/8	4 (2.0)	0/4	3 (1.0)	0/5	15 (1.6)	0/17	0.0
Tioga	5	5 (3.4)	0/5	4 (3.0)	0/4	3 (1.3)	0/4	12 (2.7)	0/13	0.0
All in one	4	4 (2.2)	0/4	3 (1.3)	0/3	3 (1.0)	0/3	10 (1.6)	0/10	0.0
Tardy Nonpareil	3	3 (2.7)	0/3	3 (2.6)	0/3	2 (2.0)	0/3	8 (2.5)	0/9	0.0
Peerless	4	4 (2.0)	0/4	2 (1.5)	0/2	2 (1.0)	0/2	8 (1.6)	0/8	0.0
Wawona	3	3 (2.7)	0/3	2 (2.0)	0/2	2 (1.0)	0/2	7 (2.0)	0/7	0.0
Marcona	2	2 (2.5)	0/2	2 (1.5)	0/2	2 (1.0)	0/2	6 (1.7)	0/6	0.0
Ne plus ultra	2	2 (2.0)	0/2	1 (4.0)	0/1	1 (3.0)	0/1	4 (2.7)	0/4	0.0
Sonora	2	2 (2.0)	0/2	1 (4.0)	0/1	1 (2.0)	0/1	4 (2.5)	0/4	0.0
Total	198	195 (3.0)	9/195	160 (2.6)	6/160	127 (1.6)	6/143	482 (2.4)	21/498	4.2

None of the almond cultivars showed Sharka symptoms.

% Positives = percentage of positive reverse-transcription (RT) polymerase chain reaction (PCR) results after the three phenotyping cycles; N = number of replicates correctly evaluated; Symptoms = number of infected 'GF305' rootstocks (mean symptom intensity scored from 0 to 5); RT+/Total = number of positive RT-PCR results over the total number of plants correctly evaluated.

The resistance observed in some almond cultivars such as Sonora, Ne Plus Ultra, Marcona, Wawona, Peerless, or Tardy Nonpareil, should be considered as preliminary because the number of final observations was less than 10, thus necessitating additional tests.

Evaluation of rootstocks grafted onto infected GF305

Sixty-six plants from nine rootstocks with three to 12 replicates per rootstock were studied (Table 5). All grafted rootstocks evaluated were susceptible to PPV and generally showed Sharka symptoms (Fig. 3) that were confirmed by positive RT-PCR results. Among the 172 observations, 96 (55.8%) were positive RT-PCR results. The analysis showed that 'Tetra' ('Empyrean 3'), 'Penta' ('Empyrean 2'), and 'Nemaguard' were the most susceptible. The important differences between cycles were remarkable, reaching an infection rate more than 90% in the first cycle because most of the replicates displayed Sharka symptoms (55 plants). This finding was in contrast to the significant decrease in plants that showed Sharka symptoms during the following cycles (13 plants and 12 plants). In general, the viral titer decreased during the second and third cycles, both in the 'GF305' rootstock and in the grafted genotypes.

During the second cycle, 'Nemaguard', 'Viking', 'Citation', and 'Guardian' showed no symptoms; in the case of Guardian, the PCR results were negative for all six replicates tested. During the third cycle, 'Viking', 'Lovell', 'Citation', 'Guardian', and 'Hansen 536' did not display symptoms, and most of the replicates had negative RT-PCR results despite the clear symptomatology shown by the 'GF305' rootstock.

Evaluation of rootstocks growing on their own roots

During this assay, 151 plants of 10 rootstocks were studied with approximately 15 replicates per rootstock (Table 6). 'Adesoto' and 'GF305' were very susceptible. 'Marianna 26/24' and 'Rootpac-R' showed less symptomatology, but the number of infected plants was high. 'Garnem' showed susceptibility to PPV, with 56.9% of plants showing positive PCR results. However, 'GF677' and 'DryStock™ One' never showed symptoms and PPV was not detected by the PCR.

Overall, no decrease in symptomatology was observed across cycles, although none of the 18 'Cadaman' replications showed Sharka symptoms in the third cycle and only one had positive RT-PCR results. Among the 443 PCRs performed, the virus was detected in 223 (50.3%), with a mean intensity of symptoms of 2.6.

Discussion

This work represents one of the most comprehensive PPV evaluations performed for *Prunus*, with 415 plants tested and 1113 PPV phenotypic observations recorded (by RT-PCR). No previous study of PPV reported such numbers, mainly because of the difficulty phenotyping Sharka under control conditions. This fact was confirmed by the high number of almond replicates lost (25%) after three phenotyping cycles. The main losses were related to fungal attacks after cold treatment (artificial winter) caused by the intense pruning, thus implying severe mechanical damage.

The inoculation success on 'GF305' was higher (95%) than that reported by a previous study (89%) (Rubio et al. 2009) that used the same PPV isolate and methodology.

There were no clear differences between the methodology used (grafted vs own-rooted). In previous studies, own-rooted plants were easier to establish and phenotype (Rubio et al. 2013) because we did not have to germinate, inoculate, and graft onto 'GF305' peach seedlings. However, this method implies lower inoculum pressure (Rubio et al. 2009) and requires performing the inoculation with infected buds (instead of a piece of bark) to verify that plants are infected (Rubio et al. 2008), particularly in those cases with resistant genotypes. Therefore, we re-inoculated all replicates of own-rooted plants that did not display Sharka symptoms initially to guard against the possible failure of the initial inoculation.

We infected 11 almond cultivars for the first time with the isolate 3.30RB/GF-IVIA and confirmed the recent findings of Rogers et al. (2024), who demonstrated that Tuono and Mission cultivars can be infected by the US isolate PPV-D (Penn4) and that Tuono is a transmission-competent host infecting healthy GF305 by aphids that had fed on diseased almonds. Among the infected almonds 'Nonpareil', 'Padre', 'Mission', and 'Price Cluster' were previously classified as resistant (Martínez-Gómez et al. 2004; Rubio et al.

Table 5. Evaluation of the behavior of rootstocks grafted onto infected 'GF305' against a plum pox virus Dideron (PPV-D) isolate sorted by susceptibility.

		Cycle 1			Cycle 2			Cycle 3			Global			
		GF305	Rootstock		GF305	Rootstock		GF305	Rootstock		GF305	Rootstock		
Rootstock	N	Symptoms	Symptoms	RT+/Total	Symptoms	Symptoms	RT+/Total	Symptoms	Symptoms	RT+/Total	Symptoms	Symptoms	RT+/Total	% Positives
Tetra (Empyrean 3)	3	3 (4.0)	3 (2.6)	3/3	3 (4.7)	3 (2.3)	3/3	2 (3.5)	2 (1.5)	2/2	8 (4.1)	8 (2.2)	8/8	100.0
Penta (Empyrean 2)	6	6 (3.0)	5 (2.0)	5/6	5 (3.4)	5 (2.8)	5/5	3 (1.7)	5 (2.6)	5/5	14 (2.9)	15 (2.5)	15/16	93.8
Nemaguard	5	5 (2.6)	5 (2.0)	5/5	4 (1.2)	0 (0.0)	2/4	3 (1.7)	3 (2.3)	3/4	12 (1.9)	8 (2.1)	10/13	76.9
Viking	7	7 (4.3)	5 (1.8)	7/7	6 (1.7)	0 (0.0)	1/6	6 (2.8)	0 (0.0)	2/6	19 (3.0)	5 (1.8)	10/19	52.6
Lovell	12	12 (3.9)	12 (1.9)	12/12	9 (2.0)	3 (1.3)	3/9	9 (2.9)	0 (0.0)	0/9	30 (3.0)	15 (1.5)	15/30	50.0
Citation	9	9 (4.1)	6 (1.3)	8/9	9 (1.8)	0 (0.0)	4/9	9 (2.9)	0 (0.0)	1/9	27 (2.9)	6 (1.3)	13/27	48.1
Guardian	8	7 (3.8)	7 (3.2)	7/7	6 (1.5)	0 (0.0)	0/6	5 (2.4)	0 (0.0)	1/5	18 (2.6)	7 (3.2)	8/18	44.4
Hansen 536	7	6 (5.0)	6 (1.5)	6/6	5 (2.2)	1 (1.0)	2/5	7 (1.6)	0 (0.0)	0/7	18 (2.9)	7 (1.3)	8/18	44.4
Nemared	9	9 (3.8)	6 (2.5)	6/9	7 (1.5)	1 (1.0)	1/7	7 (2.7)	2 (1.0)	2/7	23 (2.8)	9 (2.0)	9/23	39.1
Total	66	64 (3.9)	55 (2.0)	59/64	54 (2.1)	13 (2.1)	21/54	51 (2.7)	12 (2.1)	16/51	169 (2.9)	80 (2.0)	96/172	55.8

% Positives = percentage of positive reverse-transcription (RT) polymerase chain reaction (PCR) results after three phenotyping cycles; N = number of replicates correctly evaluated; RT+/T = number of positive RT-PCR results over the total number of plants correctly phenotyped; Symptoms = number of infected GF305 or grafted rootstocks (mean symptom intensity scored from 0 to 5).

2003). Remarkably, several of the positive genotypes have 'Tuono' or 'Mission' in their genetic background. These new findings demonstrated the ease of infecting almond trees with Sharka and that almond trees can act as potential spreaders of PPV, thus presenting a significant challenge for the almond industry. None of the infected cultivars showed clear Sharka symptoms, thus making early disease detection difficult and indicating that the use of accurate molecular detection methods for PPV is mandatory.

Recently, almond trees were suggested as a natural host of PPV in the Trakya region of Turkey (İlbağı and Çıtır 2014). However, they detected only five positive samples out of 260, and four of them were also infected with *Prunus necrotic ringspot virus* and *Prune dwarf virus*. Similarly, a recent work also confirmed the presence of a novel isolate of PPV-T that infects almond trees in Turkey (Akbaş et al. 2023). These new findings suggest that PPV had been infecting almonds for many years but was not detected. This may be attributable to its low concentration in the tissues and its erratic distribution in the trees, as already reported for 'Marianna' plum 'GF8-1' *Prunus cerasus* (Ferri et al. 2002) and apricot (Dicenta et al. 2003), creating the possibility of latent infections and the false-negative RT-PCR results (Wetzel et al. 1991). Considering the nature of our test and the number of PPV-positive samples among the infected genotypes (21/306), we could suggest that the chance of selecting infected leaves in the field is very low because, for Sharka phenotyping, the sampling must be directed toward leaves with symptoms. Under controlled conditions in our potted trees, we collected, on average, three to five leaves among the 20 to 30 leaves that our plants had.

Regarding rootstocks, regardless of the methodology (grafted vs. own-rooted), we observed rootstock susceptibility with variability in symptom expression during the three cycles, thus corroborating the general erratic distribution of PPV in *Prunus* tissues. Only 'GF667' and 'DrystockOne' appeared to be resistant after three phenotyping cycles.

The almond seedling 'Drystock One' is one of the first almond rootstocks to be tested against PPV and confirmed the general apparent resistance displayed by almonds. 'GF677' resistance to PPV-D has been previously reported by several authors (Boeglin et al. 2006; Rubio et al. 2005, 2013; Vidal et al. 2010). In contrast, other interspecific hybrids of *P. dulcis* × *P. persica*, such as 'Garnem', showed intermediate susceptibility, thus confirming previous controlled study results of Rubio et al. (2013), but they were described as resistant by other authors based on only field studies (Cinar et al. 2022; Vidal et al. 2010). Hansen 536, another *P. dulcis* × *P. persica* hybrid, had intermediate susceptibility in our test; however, during the third cycle, none of the seven replicates previously characterized as resistant showed symptoms, and none had positive RT-PCR results (Martínez-Gómez et al. 2004). 'Cadaman' (*P. persica* × *P. davidiana*) showed some susceptibility (22%) in the first and second cycles; however, during the third cycle, none of the replicates showed symptoms and only one had positive RT-PCR results. This finding was in agreement with previous data regarding low susceptibility to PPV-D (Rubio et al. 2013); however, it differed from the findings described by others regarding PPV-D resistance (Vidal et al. 2010) and PPV-M resistance (Pólak and Oukropec 2010). Similarly, 'Cadaman' has been described as susceptible to the Marcus strain (Pascal et al. 2002; Rubio et al. 2013). 'Nemaguard' and 'Nemared' have the same genetic background (*P. persica* × *P. davidiana*) and displayed a higher level of susceptibility (76.9% and 39.1%) than that of 'Cadaman'. Both rootstocks had been previously tested by Rubio et al. (2005), and 'Nemaguard' was found to be more susceptible in the current study.

Most of the plum-type rootstocks that encompass *Prunus insititia*, *P. cerasifera*, *P. domestica*, *P. salicina*, *P. besseyi*, *P. munsoniana*, or their hybrids were highly susceptible to PPV, and many replicates displayed strong Sharka symptoms and more than 80% had positive RT-PCR results, thus confirming previous findings (Rubio et al. 2005, 2013; Vidal et al. 2010). Among this group of rootstocks, only 'Krymsk 86' and 'Rootpac 20' had fewer than 35% of infected plants.

Regarding the peach (*P. persica*) rootstocks, the 'GF305' had the great susceptibility, justifying its use as index rootstock for Sharka phenotyping. Regarding 'Lovel', we reached an infection level of 50% even though it was previously described as only slightly susceptible (11%) (Martínez-Gómez et al. 2004). The complex hybrid 'Viking' was less susceptible (52.6%) than it had been in a previous study (Rubio et al. 2013). Discrepancies between studies and the behavior of the genotypes are common in PPV phenotyping, often because of differences in the evaluation method (Rubio et al. 2008) as well as the tested strain and/or isolate (Kegler et al. 1998; Rankovic et al. 1999). Marcus isolates were generally more aggressive than Dideron isolates (Cambra et al. 2006).

Conclusions

The results and data obtained during the present work demonstrated a significant risk for Sharka in California. The main finding of this study was that a PPV-D isolate can infect many almond cultivars and most rootstocks. Rootstocks that were particularly vulnerable were those with a plum genetic background such as Adesoto, Marianna 2624, Rootpac® R, Tetra (Empyream 3), and Penta (Empyrean 2), as well as peach Nemaguard, suggesting that these rootstocks should be avoided. Infected almond material could act as a source of more extensive infection of nearby stone fruit orchards either through direct aphid transmission or through long-distant transport of nursery stock or preprocessed almond fruit. Effective control of Sharka requires growers, nursery employees, and associated field workers to remain

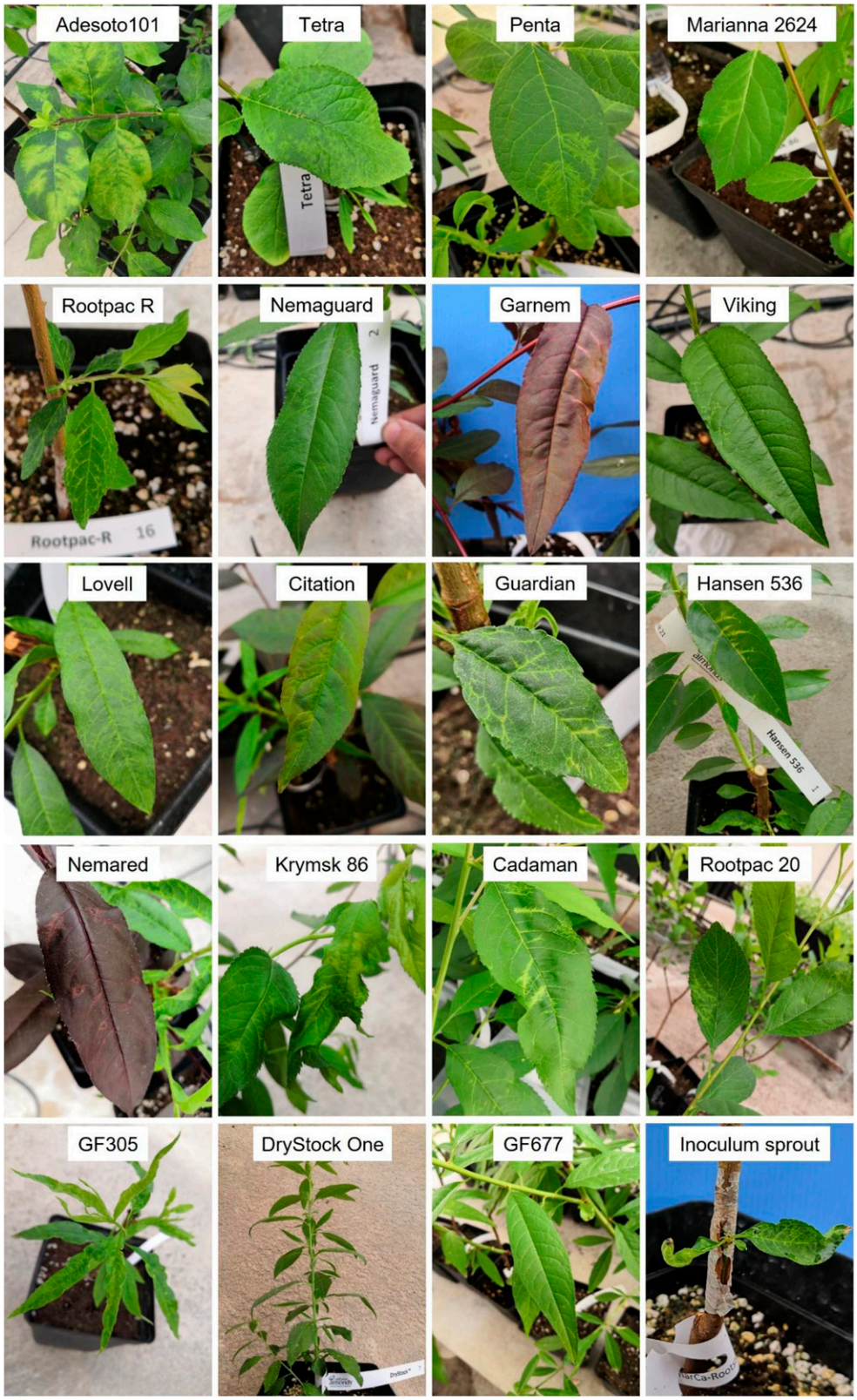

Fig. 3. Symptoms displayed by Sharka disease on rootstocks from susceptible to resistant plants. Venial chlorosis, line patterns, rings, spots, and leaf deformations are observed. The last photo shows an infected sprout that confirms that proper inoculation was performed on the own-rooted rootstocks.

vigilant for early Sharka detection and use elimination methods, including strict controls against the import of infected material. A key to such rapid detection is effective education concerning early Sharka detection based on leaf symptoms. In addition, rapid, easy, reliable, and cost-effective methods of PPV verification, such as those based on lateral flow immunochromatography (e.g., AgriStrip; BIOREBA, Reinach, Switzerland), should be adopted. Control of Sharka, once it is established, is a very difficult task; therefore, prevention is the preferred control method for this disease. Rootstocks from nurseries can play a significant role in disease spread because of their clonal propagation, high planting density, and the fact that plants in the juvenile stage are more susceptible to infection by aphids or through grafting with infected buds. Once planted in the field, direct infection of rootstocks is unlikely, except for those with a tendency to produce

Table 6. Evaluation of the behavior of own-rooted rootstocks against a plum pox virus Dideron (PPV-D) isolate sorted by susceptibility.

		Cycle 1		Cycle 2		Cycle 3		Global		
Rootstock	N	Symptoms	RT+/Total	Symptoms	RT+/Total	Symptoms	RT+/Total	Symptoms	RT+/Total	% Positives
Adesoto101	14	14 (4.2)	14/14	14 (2.9)	14/14	14 (4.3)	14/14	42 (3.8)	42/42	100.0
GF305	12	12 (3.7)	12/12	12 (3.1)	11/12	12 (4.0)	12/12	36 (3.6)	35/36	97.2
Marianna 2624	15	14 (2.3)	14/15	14 (1.8)	14/15	14 (2.0)	14/14	42 (2.0)	42/44	95.0
Rootpac-R	17	14 (3.2)	14/17	12 (1.8)	14/17	14 (2.7)	14/17	40 (2.6)	42/51	82.4
Garnem	17	9 (1.9)	11/17	6 (1.8)	9/17	9 (1.1)	9/17	24 (1.6)	29/51	56.9
Krymsk 86	13	8 (1.5)	6/13	3 (1.3)	3/13	2 (1.0)	3/11	13 (1.4)	12/37	32.4
Cadaman	18	3 (3.3)	3/18	8 (1.3)	8/18	0 (0.0)	1/18	11 (1.8)	12/54	22.0
Rootpac-20	15	3 (2.0)	3/15	4 (1.7)	4/15	2 (1.5)	2/13	9 (1.7)	9/43	20.9
GF677	15	0 (0.0)	0/15	0 (0.0)	0/15	0 (0.0)	0/15	0 (0.0)	0/45	0.0
DryStock™ One	15	0 (0.0)	0/15	0 (0.0)	0/15	0 (0.0)	0/10	0 (0.0)	0/40	0.0
Total	151	77 (2.9)	77/151	73 (2.1)	77/151	67 (2.8)	69/141	217 (2.6)	223/443	50.3%

% Positives = percentage of positive reverse-transcription (RT) polymerase chain reaction (PCR) results after three phenotyping cycles; N = number of replicates correctly inoculated; RT+/Total = number of positive RT-PCR results over the total number of plants correctly phenotyped; Symptoms = number of infected rootstocks (mean symptom intensity scored from 0 to 5).

suckers (as is common with many plum species). The production of young suckers in infected rootstocks is also a potential source of PPV transmission to nearby orchard trees by aphids.

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