Almond Board of California
Final Report-2002-2003

Project Title: **Level of Susceptibility to Plum Pox disease of California Almond Varieties**

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Location: Department of Pomology, University of California at Davis

Objective:

1. Test principal California almond varieties, rootstocks and breeding lines for susceptibility to Plum Pox disease, including the presence and (if present) nature of disease symptoms on leaves, flowers, fruit and kernels.

2. If no disease symptoms are observable, document the level (or absence) of virus in the plant cytoplasm available for spreading the disease.

3. Publish findings to provide a basis for the formulation of quarantine restrictions if (when) Plum Pox is discovered in California.

Plum pox potyvirus (PPV), the cause of the most destructive viral disease of stone fruit in Europe, (called plum pox or Sharka) has now been established in North America. It has been confirmed in Pennsylvania and also Ontario, Canada, -thus the casual import of any stonefruit wood or possibly even seed from PPV areas, including Europe and Central Asia could introduce it to California. Plum pox strains are reportedly capable of causing disease in peaches, plums, apricots, nectarines, almonds, sweet and sour cherries, as well as in other selected Prunus and non-Prunus species. It is aphid-transmitted in a non-persistent, stylet-borne manner, mechanically transmitted, and may be seed-transmitted. Movement of nursery stock and grafting also spreads the virus. Infected leaves and fruit show chlorotic (yellowing) and necrotic (browning) ring patterns, and chlorotic bands or blotches. Infected leaves and fruit also can be free from symptoms, or have symptoms that are ameliorated during the growing season. Virus infection can cause considerable losses. About 100 million stone fruit trees in Europe are estimated to be currently infected, and susceptible cultivars can result in 20-100% yield losses. Quarantine regulations were imposed between countries exchanging Prunus germplasm, which slowed the movement of the disease. Despite this effort, PPV continues to spread. In this project we have formed a collaborative program with a leading Plum Pox Research Institute (CEBAS-CSIC) in Murcia, Spain, to test the susceptibility to Plum Pox disease of 20 California almonds. The evaluation of resistance is being carried out in specialized screening facilities in Murcia, managed by cooperators P. Martinez-Gomez and F. Dicenta. Diseased (inoculated) GF305 rootstocks showing strong Sharka (PPV strain D) symptoms have been grafted with a chip-bud of the almond selections and evaluated for 2 cycles (seasons) of testing. No symptoms have been identified on any of the almond varieties tested with symptoms consistently forming on known susceptible tester stock. To further verify the absence of the virus, an ELISA-double antibody sandwich indirect (DASI) using 5B monoclonal antibodies against the virus capside protein was used. This sensitive test, as well as specially developed molecular markers for the Plum Pox virus can identify very low virus levels even when symptoms are absent. Final results demonstrate the absence of the virus in almonds but its presence in peach, including peach rootstocks and some almond x peach breeding lines (Table 1). Ten additional almond varieties and selections (which together with the first selections are responsible for approximately 95% of present California production) have now completed testing with similar results (Table 1). The second cycle of evaluation was completed in January, 2003. Full results and detailed description of materials and methods have been published in the Journal of the American Society of Horticultural Science, the leading international journal in this area. This publication is attached as a PDF file. The complete citation is:

Martínez-Gómez, P., Rubio, M., Dicenta, F., Gradziel, T.M. 2004. Resistance to Plum Pox Virus (Dideron isolate RB3.30) in a group of California almonds and transfer of resistance to peach. Journal of American Society for Horticultural Science. 129 (4): 544-548.

The verification that California almonds are immune to PPV (no symptoms, no virus in cytoplasm and so no danger of spreading the virus), is important because no control measures have yet been developed except for the complete destruction of infected orchards and the implementation of strict quarantine controls. Since European almond varieties are reported to be symptomless carriers of the disease, almond orchards may be considered for destruction despite the absence of symptoms on fruit or trees when PPV is discovered in California. The demonstration of immunity in the major California almond varieties tested in this study may allow their exemption from such quarantine restrictions. Given the inconsistent reports of susceptibility of some European almonds, presently untested California almond varieties may need to be tested before a universal immunity of California almond cultivars is concluded.

Table 1. Evaluation of PPV resistance in Californian almond cultivars

	Cycle 1				Cycle 2			
Genotype	No.	GF305**	Cultivar*	ELISA	N°*	GF305**	Cultivar	ELISA
Nonpareil	1	1	0	-	1	3	0	
Mission	4	3	0	-	3	2.66	0	
Padre	2	1.50	0	-	3	2.66	0	
Winters	2	2.50	0	-	2	3.5	0	-
F8, 5-166 PA	5	2.20	0	-	3	3	2(1)	+
F10C, 12-28 PA	4	3	0	-	4	3	0	
F10C, 20-51 PA	3	2.66	0	-	3	2	0	
F10D, 7-22 PA	1	1	0	-	1	2	0	
Halford peach rootstock	3	3.66	1(1)	+	4	1.5	0	+
54, P455 peach	5	2.80	1.5(3)	+				
F8, 5-166 PA rootstock	2	2.50	0	-				
Nickels PA rootstock	3	3.33	0	-				
Hansen PA rootstock	3	1.66	0	-				
Carmel	3	3.66	0	-				
Ne plus ultra	1	4	0	-				
Price	1	1						
Butte	0		0	-				
Sonora	0							
Lovell peach rootstock	3	2.33	2(1)	+				
Nemaguard rootstock	2	3	2(2)	+				

No. -Number of repetitions evaluated,
* Mean intensity of symptoms in the cultivar,
** Mean intensity of symptoms in the rootstock
PA Peach x Almond cross (rootstock/breeding)

J. Amer. Soc. Hort. Sci. 129(4):xxx–xxx. 2004.

Resistance to Plum Pox Virus (Dideron Isolate RB3.30) in a Group of California Almonds and Transfer of Resistance to Peach

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Additional index words. *Prunus dulcis*, *Prunus persica*, sharka, interspecific hybridization, germplasm, ELISA-DASI, RT-PCR

Abstract. Sharka [(plum pox virus (PPV)] mainly affects *Prunus* species, including apricot (*Prunus armeniaca* L.), peach (*Prunus persica* L.), plum (*Prunus salicina* Lindl., *Prunus domestica* L.) and to a lesser degree, sweet (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.). Level of resistance to a Dideron isolate of PPV in seven California almond [*P. dulcis* (Miller) D.A. Webb], five processing peach cultivars, and two peach rootstocks was evaluated. In addition, almond and peach selections resulting from interspecific almond x peach hybridization and subsequent gene introgression were tested. Evaluations were conducted in controlled facilities after grafting the test genotypes onto inoculated GF305 peach rootstocks. Leaves were evaluated for PPV symptoms during three consecutive cycles of growth. ELISA-DASI and RT-PCR analysis were also employed to verify the presence or absence of PPV. Peach cultivars and rootstocks showed sharka symptoms and were ELISA-DASI or RT-PCR positive for some growth cycles, indicating their susceptibility to PPV. Almond cultivars and almond x peach hybrids did not show symptoms and were ELISA-DASI and RT-PCR negative, demonstrating resistance to PPV. Two (almond x peach) F_2 selections as well as two of three backcrossed peach selections also showed a resistant behavior against the PPV-D isolate. Results demonstrate a high level of resistance in almond and indicate potential for PPV resistance transfer to commercial peach cultivars.

Sharka, as caused by plum pox virus (PPV), is a serious disease of temperate fruit production. PPV affects most *Prunus* species, resulting in severe economic losses in apricot (*Prunus armeniaca*), plum (*Prunus salicina*, *Prunus domestica*) and peach (*Prunus persica*) (Németh 1994). PPV has also recently been detected in sweet cherry (*Prunus avium*) (Creszenci et al., 1997). Described for the first time in Bulgaria in 1917, sharka spread throughout Europe, North Africa, India and Chile (Németh, 1994), and more recently to North America (Levy et al., 2000). PPV is characterized by a high genetic variability. Two major strains, Dideron (PPV-D) and Marcus (PPV-M), exist in Western Europe (Candresse et al., 1994). Other less common PPV isolates include El Amar (PPV-E) in North Africa and Cherry (PPV-C) in Central Europe (Kölber, 2001). To date, only PPV-D isolates have been detected in North (United States and Canada) (Damsteegt et al., 2001) and South America (Chile) (Reyes et al., 2001).

Because of its rapid transmission by aphids, sharka is difficult to control (Németh, 1994). Short-term field control methods include the removal of diseased trees and planting certified virus-free material. The development and cultivation of resistant cultivars, however, may be the only long-term solution. The development of resistant genotypes and the associated search for sources of resistance to sharka are the two of most important objectives of *Prunus* improvement programs in Europe, including apricot (Audergon et al., 1994; Egea et al., 1999), plum and prune (Dosba et al., 1994; Kegler et al., 1994) and peach (Gabova, 1994; Pascal et al., 2003).

Received for publication 13 June 2003. Accepted for publication 11 Dec. 2003. We gratefully acknowledge the assistance of Mariano Gambín in experimental work. This study was supported by funds from the Almond Board of California, California Association of Nurserymen, and the California Cling Peach Advisory Board.
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Peach and almond [*P. dulcis* (Miller) D.A. Webb syn. *P. amygdalus* Batsch] represent consanguineous species which evolved under two distinct environments, being warmer and more humid in the case of peach, and colder and xerophytic for almond (Watkins, 1976). Gradziel et al. (2001) and Gradziel (2003) have previously demonstrated the use of almond germplasm for peach improvement. No source of PPV resistance has been described in *P. persica* to date (Escalettes et al., 1998; Gabova, 1994; Pascal et al., 2003), though almond has been described as a nonhost species (Németh, 1994, Kölber, 2001). Resistance to PPV in some almond cultivars has been described (Dicenta et al., 2002; Pascal et al., 2003; Rubio et al., 2003). Pribék et al. (2001), however, previously described the presence of a Type Dideron isolate infecting almond plants, and Dallot et al. (1997) also reported experimentally infecting the 'Aï' almond cultivar by graft-inoculation.

In this study, PPV-D resistance for several Californian almond and peach cultivars, interspecific almond x peach hybrids and selfed and backcrossed progeny was evaluated under controlled conditions using both visible leaf symptoms as well as molecular probes for disease identification.

Materials and Methods

Plant materials. Plant material evaluated included seven almond cultivars, five processing peach cultivars, and two peach rootstocks (Table 1). Also tested were genotypes resulting from interspecific almond x peach hybridization as well as subsequent backcrossing and selfing with selection for peach fruit types.

PPV isolate. PPV isolate RB3.30 was used as virus inoculum and is a Dideron Type isolate obtained in Spain from the plum 'Red Beaut'. The isolate is maintained at the Instituto Valenciano de Investigaciones Agrarias (IVIA) Valencia, Spain (Asensio, 1996).

Table 1. Plant material evaluated including the origin, use, and main horticultural characteristics; BC = backcrossed.

			Horticultural characteristic			
Genotype	Origin	Use	Endocarp	Mesocarp color	Mesocarp texture	Flowering time
Carmel	Nonpareil x Mission	Almond	Paper	White	Almond-like	Very early
Mission	Early California selection	Almond	Hard	White	Almond-like	Early
Ne Plus Ultra	Early California selection	Almond	Soft	White	Almond-like	Very early
Nonpareil	Early California selection	Almond	Paper	White	Almond-like	Early
Padre	Mission x Swanson	Almond	Hard	White	Almond-like	Early
Price	Nonpareil x Mission	Almond	Paper	White	Almond-like	Early
Sonora	Nonpareil x Eureka (BC)	Almond	Paper	White	Almond-like	Very early
Andross	Early California selection	Processing peach	Hard	Yellow	Peach-like	Late
Bolinha	Brazilian selection	Processing peach	Very hard	Yellow	Peach-like	Early
Dr Davis	California selection	Processing peach	Hard	Yellow	Peach-like	Late
Halford	Lovell seedling	Processing peach	Very hard	Yellow	Peach-like	Late
Ross	California selection	Processing peach	Very hard	Yellow	Peach-like	Late
Lovell	Early California selection	Peach rootstock	Very hard	White	Peach-like	Late
Nemaguard	*P. davidiana* x peach	Peach rootstock	Very hard	White	Intermediate	Early
Hansen 536	Almond x peach	Hybrid rootstock	Very hard	White	Intermediate	Very early
Nickels	Almond x Nemaguard	Hybrid rootstock	Very hard	White	Intermediate	Early
54P455	Peach selection	Peach breeding line	Hard	Yellow	Peach-like	Early
7926-1	Padre almond x 54P455	Hybrid breeding line	Very hard	White	Intermediate	Early
F10C,20-51	(Padre x 54P455) F_2	Almond breeding line	Paper	White	Peach-like	Early
F10C,12-28	(Padre x 54P455) F_2	Almond breeding line	Paper	White	Peach-like	Early
F8,5-156	(Peach x F10C,12-28) F_2	Peach breeding line	Very hard	Yellow	Peach-like	Early
F8,5-166	(Peach x F10C,12-28) F_2	Peach breeding line	Very hard	Yellow	Peach-like	Early
99,15-154	(Peach x Nonpareil) BC_2	Peach breeding line	Very hard	Yellow	Peach-like	Late

Resistance evaluation procedure. Evaluation experiments were carried out in a sealed greenhouse in Murcia (Spain), following procedure described by Martínez-Gómez and Dicenta (1999). Scions were propagated onto infected symptomatic GF305 peach seedlings (one scion per seedling). GF305 peach is characterized by its susceptibility to PPV (Bernhard et al., 1969). Following 4 months of growth, scion-grafted trees were forced into dormancy by subjecting them to 7 °C and darkness for 2 months. After this cold dark treatment, trees were moved to an insect-proof greenhouse for 4 months. Three cycles of evaluation were performed over 2 years. The number of plants evaluated depended on scion graft success as only plants where the GF305 rootstock showed unambiguous PPV symptoms were considered as successfully inoculated. During each growth cycle leaf symptoms were scored from 0 (no symptoms) to 5 (maximum intensity of symptoms as observed on GF305 rootstock) at 2 months following budbreak. PPV symptoms evaluated include chlorotic discoloration of expanding and mature leaves and deformations of leaf tips and margins (Fig. 1). ELISA-DASI or RT-PCR positive reactions and the presence of disease symptoms in leaves in any cycle indicated the susceptibility of the genotype.

ELISA-DASI analysis. To ascertain the presence or absence of PPV in samples, an ELISA-DASI (Double Antibody Sandwich Indirect) assay was applied to the leaves during the first and third growth cycles using the 5B monoclonal antibody against the coat protein of PPV (Cambra et al., 1994). Optical densities (OD) at 405 nm were recorded after 60 min. In accordance with Sutula et

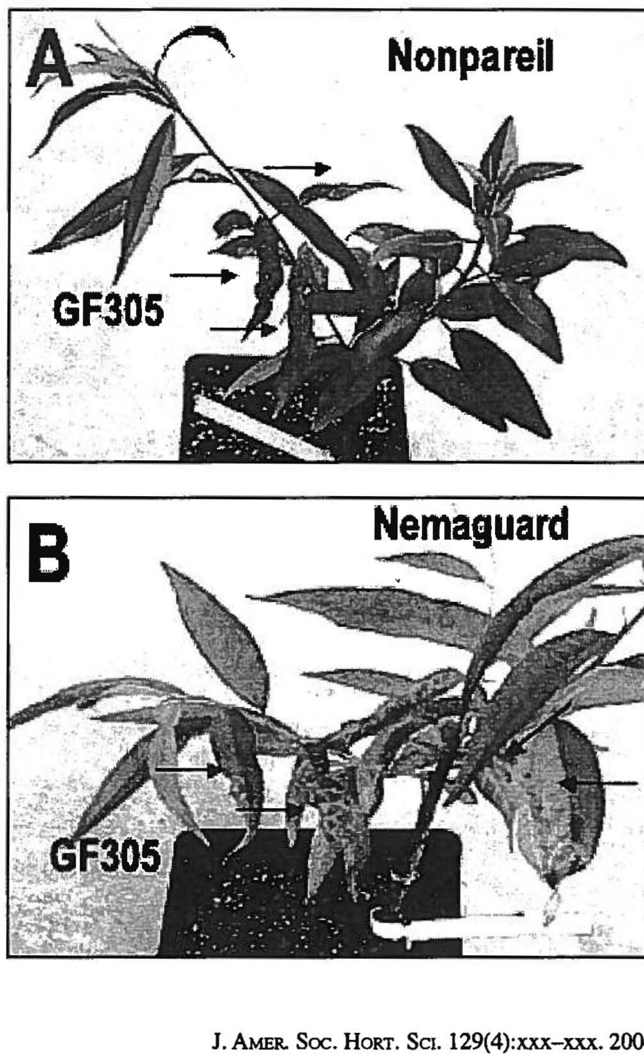

Fig. 1. Disease response following grafting onto PPV infected rootstock. (A) Absence of PPV symptoms in *Nonpareil* almond grafted onto *GF305* showing strong symptoms of the disease (indicated by arrows). (B) PPV symptoms (indicated by arrows) in the *Nemaguard* peach grafted onto *GF305* also showing symptoms of the disease.

al. (1986), samples with OD at least double those of the healthy control were considered ELISA-positive.

RT-PCR analysis. RT-PCR analysis (Wetzel et al., 1991) was carried out using total RNA extracted using the Rneasy Plant Mini Kit (Quiagen, Valencia, Calif.) as described by MacKenzie et al. (1997). Two specific primers within the coat protein (CP) gene, VP337 (CTCTGTGTCCTCTTCTTGTG) complementary to 9487-9508 positions of genomic PPV and VP338 (CAATAAAGCCATTGTTGGATC) homologous to 9194 to 9216 positions, were assayed. PCR parameters were: one cycle at 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and finally with an extension temperature at 72 °C for 5 min (Martínez-Gómez et al., 2003a). Amplified products were electrophoresed in 1% agarose gels in 40 mM Tris-acetate and 1 mM EDTA, pH 8.0 (TAE) and stained with ethidium bromide. A 1-kb plus DNA Ladder (Invitrogen Life Technologies) was used as molecular size standard.

Results

All almond cultivars grafted onto previously inoculated GF305 peach rootstocks showed resistance to the PPV-D isolate assayed after three cycles of study (Table 2). They did not show any symptoms and were ELISA-DASI and RT-PCR negative (Fig. 2) despite the symptoms observed in the GF305 rootstock.

Processing peach cultivars Andross, Bolinha, Dr. Davis, Halford, and Ross, peach breeding parent '54P455', and the peach rootstocks Lovell and Nemaguard, were susceptible to the PPV-D isolate assayed. Symptomatic plants developed chlorotic discoloration and distortion of leaves characteristic of PPV (Fig. 1) and assayed positive by ELISA-DASI or RT-PCR during at least one of the three growth cycles assayed (Table 2).

Interspecific almond x peach hybrids, including the 'Hansen 536' and 'Nickels' rootstocks, and the ('Padre' x '54P455' peach) hybrid '7926-1', demonstrated resistance. Six of the eight almond x peach derived genotypes also showed a resistant response to the PPV-D isolate assayed. Peach-type selection 'F8,5-156' and almond breeding selections 'F10C,12-28', and'F10C,20-51', showed a resistant behavior toward PPV (Table 2, Fig. 2). Plants appeared normal for three growth cycles and tested negative by ELISA-DASI and RT-PCR. Peach breeding lines '99,15-154' and 'F8,5166', developed PPV symptoms.

Symptomatic plants were always associated with high ELISA-DASI OD values (Table 2). Four of the symptomatic and ELISA-DASI positive genotypes also gave positive RT-PCR responses while three other symptomatic ELISA-DASI positive genotypes gave negative RT-PCR responses.

Table 2. Evaluation of resistance of genotype assayed to plum pox virus (PPV)-D isolate RB3.30 of PPV.

		Cycle 1				Cycle 2		Cycle 3				
		Symptoms		ELISA-DASI		Symptoms		Symptoms		ELISA-DASI		
Genotype	Plants evaluated	Symptomatic plants	Mean intensity of symptoms[z]	Positive plants	OD_{405}[y]	Symptomatic plants	Mean intensity of symptoms	Symptomatic plants	Mean intensity of symptoms	Positive plants	OD_{405}	RT-PCR[x]
Almond												
Carmel	3	0	0	0	0.06	0	0	0	0	0	0.06	–
Mission	4	0	0	0	0.10	0	0	0	0	0	0.06	–
NePlusUltra	1	0	0	0	0.07	0	0	0	0	0	0.06	–
Nonpareil	3	0	0	0	0.10	0	0	0	0	0	0.06	–
Padre	2	0	0	0	0.09	0	0	0	0	0	0.06	–
Price	1	0	0	0	0.08	---	---	---	---	---	---	---
Sonora	1	0	0	0	0.10	0	0	0	0	0	0.05	–
Peach												
Andross	4	0	0	0	0.11	0	0	1	1.0	1	0.35	+
Bolinha	3	1	1.0	1	0.40	0	0	0	0	0	0.06	–
Dr. Davis	5	2	1.0	2	0.63	0	0	1	1.0	1	0.20	–
Halford	3	1	1.0	1	0.59	0	0	1	1.0	1	0.18	+
Ross	4	0	0	0	0.09	1	1.0	1	1.0	1	0.18	–
Peach rootstock												
Lovell	3	1	2.0	1	1.14	0	0	0	0	0	0.06	–
Nemaguard	2	2	2.0	2	1.89	0	0	1	1.0	1	0.20	–
Hybrid rootstock												
Hansen 536	3	0	0	0	0.06	0	0	0	0	0	0.06	–
Nickels	3	0	0	0	0.06	0	0	0	0	0	0.06	–
Breeding lines												
54P455	5	3	1.5	3	0.71	0	0	3	1.5	3	0.46	+
7926-1	1	0	0	0	0.07	0	0	0	0	0	0.07	–
F10C,20-51	3	0	0	0	0.08	0	0	0	0	0	0.06	–
F10C,12-28	4	0	0	0	0.08	0	0	0	0	0	0.06	–
F8,5-156	2	0	0	0	0.06	0	0	0	0	0	0.06	–
F8,5-166	5	0	0	0	0.10	1	2.0	0	0	0	0.05	–
99,15-154	3	1	2.0	1	1.68	0	0	1	1.0	1	0.27	+

[x]Positive (+) or negative (–) reaction.
[y]OD_{405} = optical density at 405 nm values after 60 min. Mean OD_{405} in infected and healthy GF305 peach rootstocks were 1.80 and 0.07, respectively.
[z]Intensity: 0 = no symptoms to 5 = maximum intensity of leaf chlorosis and distortion. Mean intensity of PPV symptoms in infected GF305 peach rootstocks was 3.0.

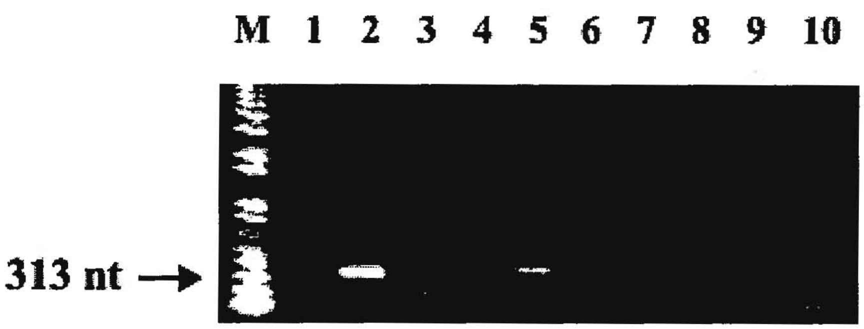

Fig. 2. Amplification products (313 bp) indicative of the presence of plum pox virus (PPV) obtained using RT-PCR for PPV detection in different samples. Lane 1 = healthy GF305 rootstock, lane 2 = GF305 rootstock infected by PPV and showing strong sharka symptoms, lane 3 = 'Hansen 536', lane 4 = 'Nonpareil', lane 5 = '54,P455' peach, lane 6 = 'Mission', lane 7 = 'Ne Plus Ultra', lane 8 = 'F10C,12-28', lane 9 = 'F10C,20-51', and lane 10 = 'F8,5-156'. Lane M = molecular weight marker 1 kb plus DNA ladder.

Discussion

Results demonstrate the susceptibility of the peach cultivars and peach rootstocks assayed. These findings agree with previous studies reporting the absence of resistance to PPV in peach (Escalettes et al., 1998; Gabova, 1994; Pascal et al., 2003). While Escalettes et al. (1998) have suggested resistance in some ornamental peach selections, the possible interspecies origin of these selections was not ruled out.

Susceptibility was observed in the 'Nemaguard' seedling rootstock, a probable progeny of a cross between peach and *Prunus davidiana* (Carr.) Franch. (Martínez-Gómez et al., 2003b). Pascal et al. (2003) reported resistance in several *P. davidiana* lines to a PPV-M isolate which was different from the PPV-D isolate used in this study.

Mean intensity of PPV symptoms of all the infected peach genotypes was of 1.4. This intensity is very low in comparison to the mean intensity of ≈3.0 observed in the infected GF305 rootstock, confirming the high susceptibility described for this rootstock (Bernhard et al., 1969). In addition, the intensity of symptoms is lower than the mean values of ≈2.0 observed in a previous evaluation of apricot cultivars with this PPV-D isolate, (Martínez-Gómez and Dicenta 2000). These results confirm the lower level of susceptibility of peach to PPV-D isolates in comparison to apricot (Kölber, 2001; Quiot et al., 1995), with the notable exception of the GF305 peach rootstock.

The very irregular distribution and the low concentration of PPV described in *Prunus* tissues (Albrechtova, 1986; Audergon et al., 1989) would result in an irregular manifestation of PPV symptoms as observed in the replications of each cultivar during the three studied cycles. In many cases, some of the inoculated replications of a given cultivar showed PPV symptoms while others did not. This irregular distribution has important implications for virus detection, since it means that plants which are really infected may appear healthy (Marenaud and Yürektürk, 1974; Desvignes, 1976, Quiot et al., 1995). In this evaluation, we considered that genotypes were susceptible to PPV that showed PPV symptoms or were positive by ELISA-DASI or RT-PCR during any of the growth cycles assayed. This more conservative screening strategy has given consistent results in previously reported disease studies (Dicenta et al., 2003; Martínez-Gómez and Dicenta, 1999, 2000; Martínez-Gómez et al., 2003a). In our assays, the first and the third cycles of study were performed during the spring, whereas the second cycle of study was performed at the end of summer when temperatures were higher. Hubert et al. (1988) observed that high temperatures reduce the manifestation of PPV symptoms in *Prunus*. Higher temperatures, thus, may have contributed to the lower PPV symptoms observed during the second cycle of the study.

All plants showing symptoms also gave positive ELISA-DASI readings. In addition, detection of PPV by RT-PCR during the third cycle confirmed the results obtained by ELISA-DASI. All samples that were positive by RT-PCR were also positive by ELISA-DASI. However, in the cases of lower OD of ELISA-DASI (peach cultivars Dr. Davis and Ross, and Nemaguard rootstock), the RT-PCR was negative. While a higher sensitivity has been reported for the RT-PCR in comparison to the ELISA-DASI (Candresse et al., 1994; Martínez-Gómez et al., 2003a; Wetzel et al., 1991), the erratic distribution of PPV common in infected *Prunus* tissue together with the presence of PCR inhibitors described by Olmos et al. (2002) in some *Prunus* tissues could have contributed to false RT-PCR negatives.

The level of resistance to the PPV-D isolate of all the California almond cultivars assayed support Dicenta et al. (2003), Pascal et al. (2003), and Rubio et al. (2003), who reported the resistance of selected European almond cultivars to PPV-D and PPV-M isolates. Dallot et al. (1997), detected the PPV virus by ELISA in 'Aï' almond cultivar, after graft-inoculation with 5 Dideron, 3 Marcus and 1 El Amar isolates. However, the ODs they obtained were low, particularly in the almond inoculated with PPV-D isolates. Only one isolate induced some chlorotic discoloration of the leaves of 'Aï', which rapidly disappeared. Dallot et al. (1997) also demonstrated a lower rate of infection by PPV-D isolates, as described previously by Quiot et al. (1995) in apricot and peach. While 'Aï', may represent a particular case of susceptibility among almond cultivars, Dallot et al. (1997) did not find any ELISA-positive samples after analyzing 356 trees in a field survey.

Results support the low potential of the almond genotypes used in this study as virus sources in sharka epidemics where Type D isolates of PPV are involved. The almond cultivars used in this study represent ≈70% of current production in California, with most remaining commercial varieties being the progeny of crosses between the resistant 'Nonpareil' and 'Mission' varieties (Martínez-Gómez et al., 2003b). Type D, which is the most readily transmitted isolate, is the major isolate found in Western Europe and the only isolate reported in North and South America (Damsteegt et al., 2001). In both Western Europe and North America, the control of PPV is through widespread and recurrent visual and ELISA-DASI based surveys of existing orchards with tree removal and quarantine restrictions when PPV is found. Confirmation of a freedom from PPV in remaining almond cultivars planted in California could lead to the exclusion of these almond varieties as a potential virus reservoir. In California, where plantings of these almond varieties account for ≈180,000 ha, their removal as a potential host species would allow a more efficient focusing of virus surveys to susceptible *Prunus* crops.

These findings support the hypothesis that transfer of some level of PPV resistance from almond to peach breeding lines is possible. All almond x peach hybrids as well as six of the eight genotypes derived from interspecific hybridizations were resistant to PPV. The absence of any formidable crossing barriers in either the initial hybridization or subsequent backcrosses between peach and almond (Gradziel et al., 2001; Gradziel, 2003) further supports the suitability of almond germplasm for peach improvement. Two resistant breeding lines, 'F10C,20-51' and 'F10C,12-28' have an almond-type tree and nut, and were selected for their high level of self-compatibility derived from the peach parent. The resistant selection 'F8,5-156' and the susceptible selection 'F8,5-166' have a peach-type tree and fruit and were selected for good canning quality and uniform fruit ripening within the tree. All selections resulted from interspecific hybridization between the resistant 'Padre' almond and the susceptible '54P455' peach. The quarantine safeguards required for PPV testing limited the number of peach and almond selections for this initial evaluation. The selections '54P455' peach, '7926-1' interspecific hybrid, and derived progeny were selected for testing since this was the population used for developing the genetic map for peach and almond (Bliss et al. 2002; Foolad et al. 1995) and our eventual goal is to map the resistance gene(s) in almond. The lack of native sources of resistance within peach (Escalettes et al., 1998; Dosba et al., 1994; Gabova, 1994; Pascal et al., 2003) also make almond species a valuable source of PPV resistance for peach species, as previously proposed by Gradziel (2003) and Pascal et al. (2003). Pascal et al. (2003) have reported resistance to PPV Type M in several *P. davidiana* lines but Moing et al. (2003) had indicated that poor fruit quality is transmitted from *P. davidiana* to peach, which was not a problem in advanced almond-derived peach selections (Gradziel, 2003).

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