Almond Board of California
Project Report-2001-02

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

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Cooperating Personnel: P. Martinez-Gomez and F. Dicenta.

Location: Department of Pomology, University of California at Davis
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Objective:

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

2. Document the level (or absence) of virus in the plant cytoplasm available for spreading the disease (by grafting, aphid transmission, etc.).

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

Background and Results

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, and was recently reported to be in Chile, -thus the casual import of any stonefruit wood or possibly even seed from these 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 can also spread the virus. The severity and relentless spread of the disease in Europe has led to the development of the Sharka International Working Group which allowed coordination of research and a free flow of information among countries. In *Prunus*, plum pox virus symptoms appear on leaves, fruits, flowers, and seeds. The severity of the symptoms varies according to the Prunus species and cultivar, PPV strain, season and location. Flowers may show a red/white mottling (Fig. 1), Leaves (Fig. 2), fruit (Fig. 3) and seed (Fig. 4) show chlorotic

(yellowing) and necrotic (browning) ring patterns, and chlorotic bands or blotches. Leaves, flowers and fruit can also be absent of 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. No control measures have yet been developed except for the complete destruction of infected orchards and the implementation of strict quarantine controls.

Figure 1. Plum pox on peach flowers.

European fresh-market and processing peach and apricot varieties have been found to be susceptible, though resistant apricot germplasm has recently been identified within US germplasm. Since European almond varieties are thought 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 nearby orchards. Recent observations by a Spanish colleague (P. Martinez-Gomez) working with my program suggest that the California almond variety *Nonpareil* may be immune to PPV (no symptoms, no virus in cytoplasm and so no danger of spreading the virus), which could make it exempt from quarantine restrictions. As *Nonpareil* is a parent to most important California almond varieties as well as several advanced processing peach breeding lines, levels of PPV resistance, if verified, may be found in other almond varieties and breeding lines as well. If plum pox resistance is verified in peach breeding lines derived from peach x almond crosses, it will be the only known source of resistance in peach at this time.

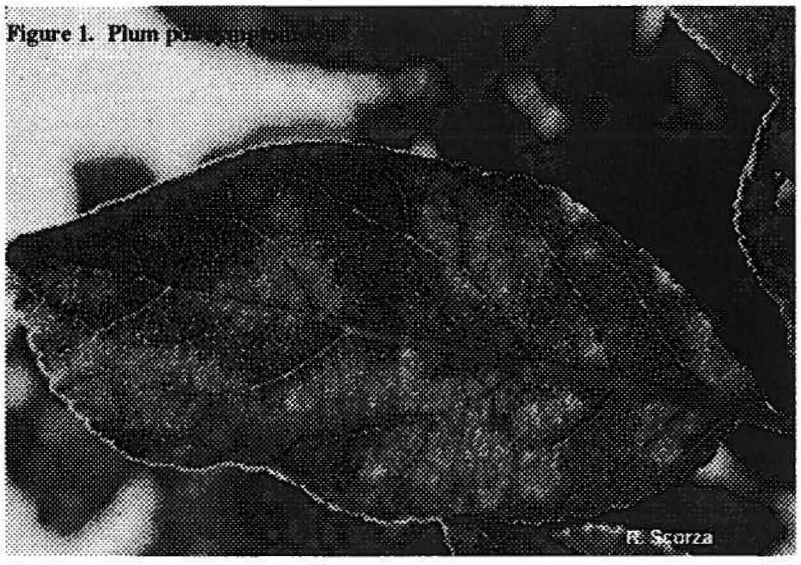

Figure 2. Plum pox symptoms on plum leaves.

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 California cling-peach varieties including *Andross, Ross, Dr. Davis, Halford* and *Loadel*, and almond varieties including *Nonpareil, Mission, Carmel, Butte* and *Padre* and advanced almond x peach breeding lines (Table 2). (The California Cling Peach Advisory Board and the Almond Board of California jointly funded this project). 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 and cling-peach selections to be evaluated. All almond and cling-peach selections (Tables 1 & 2) have now been submitted to at least 1 artificial rest period in a cold chamber at 7 °C in darkness for six weeks (to substitute for winter dormancy) and have been transferred to a specially designed virus evaluation/containment greenhouse (Fig. 5) where the buds have now sprouted and have been inspected for symptoms. No symptoms have so far been identified on any of the almond varieties tested (Table 1) with symptoms consistently forming on known susceptible tester stock (Fig. 6) as well as the California processing peach varieties (Fig. 7). To further verify the absence of the virus, an ELISA-double antibody sandwich indirect (DASI) test is being applied to the leaves using 5B monoclonal antibodies against the capside protein of the PPV (Table 2). 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.

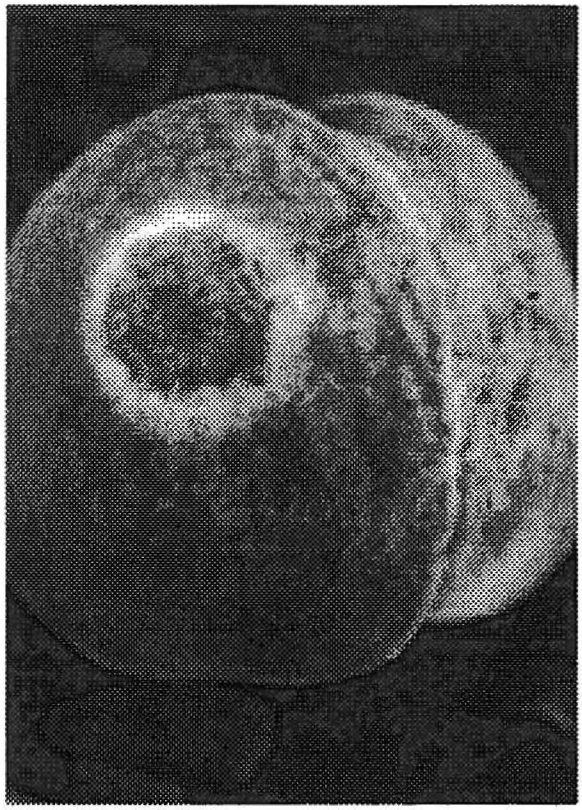

Figure 3. Plum pox on peach fruit.

Figure 4. Plum pox on apricot seed.

Table 1. Peach and almond genotypes tested including their derivation and year of first inoculation (testing).

Genotype	Derivation	Year inoculated
ANDROSS	California processing peach variety	2001
ROSS	California processing peach variety	2001
DR DAVIS	California processing peach variety	2001
HALFORD	California processing peach variety	2001
LOADEL	California processing peach variety	2002
LOVELL	California drying peach and rootstock	2002
BOLINHA	Brazilian processing peach variety	2002
RUBIDIUX	French peach variety	2002
54P455	California processing peach variety	2002
NONPAREIL	California almond variety	2001
MISSION	California almond variety	2001
PADRE	California almond variety	2001
WINTERS	California almond variety	2001
CARMEL	California almond variety	2002
SONORA	California almond variety	2002
PRICE	California almond variety	2002
BUTTE	California almond variety	2002
Ne Plus Ultra	California almond variety	2002
NEMAGUARD	Derived from peach x *P. davidiana*	2002
PADRE X 54P455	Almond x Peach hybrid	2001
NICKELS	Almond x Peach hybrid	2002
HANSEN	Almond x Peach hybrid	2002
F10C,20-51	Approximately 25% peach 75% almond	2002
F10C,12-28	Approximately 25% peach 75% almond	2002
F8,5-161	Approximately 15% almond 85% peach	2002
F8,5-166	Approximately 15% almond 85% peach	2001
99,15-145	Approximately 10% almond 90% peach	2002

Results from 2001 demonstrate the absence of the virus in almonds but its presence in peach, including peach rootstocks and some almond - peach breeding lines (Table 2). Several other almond - peach breeding lines demonstrated early signs of resistance, though these results will need to be confirmed by a second round of testing. F8,5-161 is an advanced peach-like selection, having good peach fruit and tree characteristics in addition to preliminary potential for Sharka resistance. A similar advanced selection, F8,5-166, showed susceptibility within the first year after inoculation, however, reinforcing the need for multi-year testing. The second cycle of evaluation should be complete by mid-2003.

Figure 5. UCD almond and peach selections in CEBAS Plants Quarantine Test Facility, in Murcia, Spain

The verification that California almonds are immune to PPV (no symptoms, no virus in cytoplasm and so no danger of spreading the virus), would be important because no control measures have yet been developed except for the complete destruction of infected orchards and the implementation of strict quarantine controls. The conclusive demonstration of immunity in California almond varieties may allow their exemption from such quarantine restrictions. The successful transfer of such resistance/immunity to peach would offer the only known source of plum-pox resistance within peach.

Results from 2002 confirm that all of the almond varieties and breeding lines showed a consistent lack of virus symptoms on budded shoots despite often high symptom development on the GF305 rootstock. Peach varieties, however, have typically shown symptoms within the first year. Some varieties such as Ross have shown no symptoms or ELISA positive results during the first year but have shown positive symptoms on the second cycle of growth (Table 2). Other varieties such as Halford have shown both symptoms and ELISA positive results the first year and a lack of symptoms and ELISA results in the second cycle of growth. Although seemingly contradictory, this is not unusual with the plum pox virus as it can be distributed erratically throughout the plant and at very low titers. This is why a least two cycles of growth testing and necessary before any primary conclusions can be drawn. The peach variety

Figure 6. Plum pox on GF305 rootstock prior to chip grafting Nonpareil almond.

Rubidiux, while remaining symptomless during the first cycle of growth, will probably show symptoms by cycle 2 if other peach variety experience holds. Interestingly, the Nemaguard rootstock showed symptoms despite being a putative hybrid between peach and *Prunus davidiiana* (which has been reported to be resistant). The almond X peach hybrids, including Padre X 54P455, Nichols, and Hansen, all show resistance to PPV although two cycles of testing have been completed for only the first hybrid. Peach - almond breeding lines having mostly almond germplasm with 10 to 25 percent peach germplasm continued to show the resistance associated with almond. However lines which are predominantly peach-like, and having mostly peach germplasm with 10 to 20 percent almond germplasm varied in in the resistance response. Some, such as 99,15-154, showed early and high levels of symptom development. Others such as F8,5-166 showed susceptibility, but only by the second cycle. Several, including F8,5-161 have not yet shown symptoms though the second cycle of testing has not yet been completed. The ability to transfer resistance to peach-almond hybrids is consistent with a future capacity to transfer PPV resistant genes from almond to advanced peach selections. Since many of the advanced peach breeding lines have lost most of the almond genes in the selection towards the peach type fruit and trees, the absence of resistance does nor necessarily mean that the resistance gene does not exist, but that may not yet have been located. Resistance controlled by one to a few almond genes would be relatively infrequent in these populations and so emphasizes the need to sample a large number of additional almond derived peach breeding lines. The identification of possible resistance in the Padre X 54P455 hybrid is particularly important since this was the parent for the peach-almond mapping population. If resistance is identified in any of the F2 progeny presently planted in UC orchards, the previously developed genetic linkage map might be used to develop more accurate molecular markers for this trait and so facilitate future breeding efforts.

Figure 7. Plum pox symptoms on GF305 rootstock and Andross peach.

In summary, present results support the possible immunity of almond to the plum pox virus. However a second cycle of testing needs to be completed to confirm these findings. (Testing should be completed by the summer of 2003). If confirmed, findings would have important implications for quarantine regulations when plum pox virus is found near almond orchards. Findings are also be important in identifying almond as a possible source of PPV resistance for peach. While such resistance was not been specifically identified in advanced peach breeding lines tested here, our results are very preliminary and still supportive of opportunities for successfully transferring resistance from almond to peach with continued searching within the sizable almond derived peach breeding lines presently within the UCD peach breeding program.

Table 2. Evaluation of PPV resistance in Californian almond and peach cultivars and breeding lines as characterized by symptoms on GF305 rootstock and scions over 2 growth cylces (seasons) and verified by ELISA testing for PPV virus (strain D).

	Cycle 1				Cycle 2			
Genotype	N°	GF305**	Cultivar*	ELISA	N°*	GF305**	Cultivar	ELISA
Mission	4	3	0	-	3	2.66	0	
Ross	4	2.75	0	-	4	2.50	1(1)	+
Nonpareil	1	1	0	-	1	3	0	
Andross	3	2.66	0	-	4	2.33	1	+
Dr Davis	5	2.40	1(2)	+	5	3	0	-
Halford	3	3.66	1(1)	+	4	1.5	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	
Padre x . 54,P455 PA	1	1	0	-	1	2	0	
Nickels PA	3	3.33	0	-	pending			
Hansen 536 PA	3	1.66	0	-	pending			
Carmel	3	3.66	0	-	pending			
Sonora	0				pending			
Price	1	1	0	-	pending			
Butte	0				pending			
99,15-154	3	2.66	3(1)	+	pending			
Ne plus ultra	1	4	0	-	pending			
54,P455	5	2.80	1.5(3)	+	pending			
F8, 5-161 PA	2	2.50	0	-	pending			
Rubidiux	3	2.66	0	-	pending			
Nemaguard	2	3	2(2)	+	pending			
Loadel	1	1	0	-	pending			
Lovell	3	2.33	2(1)	+	pending			
Bolinha	3	2.33	1(1)	+	pending			

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)