Identifying Factors Mediating Resistance to Almond Leaf Scorch Disease

Project Number: **07-PATH8-Kirkpatrick**

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Project Objectives:

1. Identify the biochemical or anatomical properties that eliminate *Xylella fastidiosa* infections in almond leaf scorch (ALS) resistant almond cultivars.
2. Determine if grafting a susceptible almond cultivar onto an ALS-resistant interstock can render the scions more resistant to ALS.

Interpretive Summary:

Project Rationale:
This is a new research project that just started in October 2007. Almond leaf scorch (ALS) disease is caused by the bacterium, *Xylella fastidosa* (Xf), that is transmitted in nature by several sharpshooter insect species. The rationale for this project is based on observations made by Joe Connell, UCCE Farm Advisor, Butte county in 2000 and 2001. During this period a significant outbreak of ALS, which involved hundreds of trees in several orchards, occurred in the Chico area. What was particularly striking was the relative incidence of ALS in the four almond varieties that were planted in one particular orchard. A very high incidence of ALS was found in the Peerless variety, significant disease also occurred in Nonpareil, however virtually no disease occurred in Carmel and Butte varieties, even though all of these varieties were planted in alternating rows within the orchard. This observation served as the impetus for previous research on the relative resistance to ALS in 10 commercial almond varieties, results of that research were presented at the 2005 Almond Conference and which is found in the Tables attached to this report. We found that following mechanical inoculation, Xf almond strains readily moved and caused disease in ALL almond varieties, i.e both field susceptible and field resistant varieties, in the first growing season. However, disease ratings that were made in the following 2 summers showed that several varieties emerged disease and pathogen free after going dormant the year following inoculation.

Thus the field resistance that was observed in the Chico plots was not due to the ability of a resistant variety to kill off or retard the movement of Xf during the year it was inoculated but rather resistance was a property of a particular variety to eliminate the pathogen during the winter following inoculation. This same "cold therapy" phenomenon has been observed in some grape (*Vitis vinifera*) varieties that were infected with Xf strains that cause Pierce's disease. However, what makes the study of this phenomenon in almond particularly attractive is that especially cold winter temperatures, which are required for grapevine cold curing to occur, are not required for the almond ALS curing phenomenon to occur because the varietal resistance work we did was at Davis, which has moderate winters. While we have clearly documented which varieties possess field resistance to ALS and when this resistance is expressed, we know nothing of the mechanisms involved with the winter curing phenomenon. The goal of this research project is to try and understand what varietal factor(s) mediate field resistance to ALS. An additional objective is to determine if a moderately susceptible variety like Nonpareil may become more resistant to ALS if it is grafted on an ALS-resistant interstock such as Carmel.

Research Plans for 2007-2009:

Objective 1:

Many of the procedures in Objective 1 will be similar to those we are using to better understand the biochemical basis for the cold curing phenomenon in grapes. However, we will not be exposing almonds to different temperatures which is the focus of another project being conducted by Kent Daane and colleagues and funded by the Almond Board and UC/ARS.

Two healthy ALS-susceptible varieties such as Peerless and Sonora and two ALS-resistant varieties, i.e. Butte and Carmel, will be used for the proposed biochemical and anatomical studies. Because we know that the ALS-resistance is expressed during the dormant period and these factor(s) are likely present in xylem fluids where Xf resides, we will express xylem fluids from branches using a custom made pressure bomb. Xylem sap will be expressed monthly from October, 2007 to March, 2008 as well as in July, 2008 to determine what compounds are present in xylem sap during dormant and active growing periods. pH, osomolarity, relative water content, electro conductivity, protein composition and enzyme assays, such as peroxidase activity, will be determined in our laboratory. Amino acid, organic acid and mineral composition of the sap will be determined by the UC DANR laboratory or a commercial testing laboratory. Two collections of 3 branches will be made from each tree, 2 trees will be sampled for each variety. Biochemical profiles from the susceptible and resistant cultivars will be compared. If unique proteins are noted in polyacrylamide gel electrophoresis (PAGE) profiles the unique proteins will be cut out of the gel and sequenced by the UCD Analytical Protein Laboratory to determine their identity.

The relative toxicity of the expressed xylem saps to cultured *X. fastidiosa* (Xf) cells will be determined by placing cultured Xf cells in saps from the various varieties for 4 to 24 hours after which the cells will be plated on solid PD3 medium plates. Two incubation

temperatures, 5 and 28C, will be evaluated. The number of colony forming units (CFUs) that grow on the plates will be counted and compared to Xf cells that were maintained in water or phosphate buffer at similar temperatures and for similar incubation periods.

The anatomical structure of xylem elements will be compared at 2 times during the dormancy period in January and March before bud break. Tissues will be sampled from 2.5cm diameter branches and from sections removed from the trunk. Tissues will be fixed, stained and examined by light microscopy using standard techniques. The integrity of xylem tissues collected from the time points will be compared. It is possible that the integrity of xylem tissues undergoing dormancy differs in different almond varieties. If sufficient degradation occurred it could affect the ability of Xf to overwinter in those xylem elements and possibly serve as a component of the resistance mechanism.

We hope that by comparing the biochemical and anatomical properties of 2 different almond varieties in each ALS resistance category we may be able to correlate the presence of particular anatomical properties or compounds with resistance. Such knowledge could be used as genetic markers to predict what new varieties would likely possess ALS resistance.

Objective 2:

Two different ALS-resistant varieties, Butte and Carmel, will be used as the interstock and the size of interstock will be 25", the standard size Fowler nursery normally produces. All trees would be grafted on Lovell rootstocks; interstocks would be grafted with an ALS-susceptible variety such as Peerless. Interstock trees and Peerless trees grafted on Lovell rootstocks will be Xf-inoculated, the latter being used as positive controls. Disease comparisons between the positive control and experimental trees will allow us to determine if the interstock can provide some translocatable factor that might provide protection or reduce ALS symptoms compared to standard grafted Xf-susceptible nursery trees. If the ALS-resistant interstock can confer resistance to the grafted ALS-susceptible variety, such tress could be used as replants in orchards that experience significant losses to almond leaf scorch disease.

Table 1. Incidence of almond leaf scorch (ALS) in a Chico orchard containing NonPareil, Peerless, Butte and Carmel varieties in 2002

Disease rating	NonPareil	Peerless	Butte	Carmel	Total
Healthy	794	329	217	240	1580
1	63	53	3	0	119
2	54	26	2	0	82
3	34	79	0	1	114
Total ALS trees	151	158	5	1	315
Total trees rated	945	487	222	241	1895

Table 2. Disease incidence (% ALS infected) for each almond variety in 2002

Disease rating	NonPareil	Peerless	Butte	Carmel
Healthy	84.0	67.6	97.7	99.6
1	6.7	10.9	1.4	0
2	5.7	5.3	0.9	0
3	3.6	16.2	0	0.4
Total ALS trees	**16.0**	**32.4**	**2.3**	**0.4**
Total	100	100	100	100

Disease Ratings on a 0 (healthy) to 4 (dead) scale.

Table 3. Xylella fastidiosa movement (mm) in 11 almond varieties following mechanical inoculation on 6/15/02.

Variety	**7/30**	**8/15**	**9/15**	**10/15**	**Relative Field Resistance**
Butte	0	385	705	905	High
Carmel	0	25	570	780	High
Mission	0	122	1000	1120	Low
Ne Plus	0	300	553	900	
Nonpareil	0	102	250	250	Medium
Padre	0	100	660	1035	
Peerless	0	204	808	1020	Low
Price	0	430	610	710	
Solano	0	87	672	980	
Sonora	0	120	513	700	
Thompson	0	340	670	965	

Table 4. Percentage of ALS-infected varieties 16 months after inoculation.

Variety	**ALS +/16**	**% ALS+**	**Systemic spread in tree??**	**Observed Field Resistance**
Sonora	12/16	75	++++	Very Low
Solano	8/16	50	+++	Very Low
Peerless	9/16	56	+++	Very Low
NePlus	4/16	25	+	Medium
Price	3/16	19	0	Medium
Mission	3/16	19	0	Medium
Butte	0/16	0	0	Very High
Carmel	0/16	0	0	Very High
Padre	0/16	0	0	Very High
Thompson	0/16	0	0	Very High