Identifying Factors Mediating Resistance to Almond Leaf Scorch Disease

Project No.: **08-PATH8-Kirkpatrick**

Project Leader: Bruce Kirkpatrick
Department of Plant Pathology
University of California, Davis
One Shields Avenue
Davis, CA 95616
(530) 752-2831
bckirkpatrick@ucdavis.edu

Project Cooperators: Margot Wilhelm, mwilhelm@ucdavis.edu

Objectives:

1) Identify the biochemical and anatomical properties that eliminate *Xylella fastidiosa* Almond Leaf Scorch (ALS) infections in resistant almond cultivates.
2) Determine if grafting a susceptible almond cultivar onto an ALS resistant interstock can render the scions more resistant to ALS.

Interpretive Summary:

Almond Leaf Scorch (ALS) disease is caused by the bacterium *Xylella fastidiosa* (Xf) which occupies a particular niche in plant xylem vessels. Previous work on ALS showed striking differences of disease incidence among cultivars. Disease incidence was higher in the Peerless and Nonpareil varieties while virtually no disease occurred in Carmel and Butte varieties. More recent research studying relative resistance of 10 commercial almond varieties showed that following mechanical inoculation of Xf, the bacteria readily moved and caused disease in ALL varieties. However, several of the varieties emerged disease and pathogen free after overwintering the year following inoculation.

In our current research we want to know if there are certain components of xylem sap or anatomical xylem structures that mediate resistance to ALS over winter months. Identifying differences between resistant and susceptible cultivars could point to factors useful for controlling the disease.

In the past year we extracted xylem sap from 2 ALS susceptible varieties, Peerless and Sonora, and 2 resistant varieties, Butte and Carmel. Sap was extracted in November, January, February, March and July. pH and osmolarity measurements were taken and xylem sap osmolarity clearly increases in the spring and into summer in all cultivars (Tables 1 and 2). Xylem sap composition was further analyzed for soluble calcium, magnesium, iron and sugars, fructose, glucose, and sucrose. So far, our analysis for

fructose and glucose indicates there are no significant differences among the cultivars, but these sugars increase in concentration significantly in July (Table 3). The analysis of soluble calcium indicates that calcium content changes with respect month but is not significantly different among cultivars. We also found that immediately after expression, xylem sap began to turn yellow indicating the presence of polyphenolics. Polyphenolic content of xylem sap may be important because phenolic compounds are involved in plant defenses and some phenolics can inhibit bacterial growth. Although our data are not fully analyzed, it appears that resistant cultivars may have significantly higher xylem sap polyphenolic content during autumn and winter (Table 4) which could affect Xf survival over the winter. Further results of our *in vitro* microbiological assay, described in the following paragraph, may yield more information.

Another portion of our work involves a microbiological assay in which we want to know if *X. fastidiosa* has differential survival in xylem sap from susceptible and resistant cultivars. Shorter survival in xylem sap from ALS resistant varieties would indicate that some component of resistant sap may be involved reducing Xf survival over the winter. This component could possibly be a polyphenolic compound or an anti-microbial protein. Other work in our lab showed that a particular protein present in grape xylem sap produced in response to cold temperatures is potentially involved the “cold curing” phenomenon for Pierce’s disease of grapevine. We are studying xylem sap protein profiles from our different cultivars over the sampling months to determine if a similar anti-microbial protein is present in the xylem sap of resistant cultivars.

This spring we hope to obtain almond trees with ALS resistant interstocks from Fowler nurseries. Normal, low-grafted and interstock trees with be grafted with the ALS susceptible Peerless cultivar and mechanically inoculated with *X. fastidiosa*. Disease progression and severity in the low-grafted and interstock trees will be compared over several years. It is possible that certain proteins or xylem sap components from the resistant interstock may be capable of moving into the Peerless scion and possibly conferring better ALS resistance to the entire tree.

The anatomy of xylem vessel structure could also be involved in ALS resistance or susceptibility. For example, susceptible cultivars may have smaller vessels that are more easily clogged with *X. fastidiosa* bacteria. We are comparing xylem vessel areas of healthy almond trees between one susceptible cultivar, Peerless and one resistant cultivar Butte. We will look at samples every other month throughout the winter. Almond trees produce two types of xylem vessels, larger ones produced in the spring for higher water uptake and smaller ones produced through the summer and fall. We are analyzing these two groups separately because the summer vessels have areas approximately 5-7 times smaller than spring vessels.

We anticipate that these studies may identify the factor(s) that mediate field resistance against ALS in some almond varieties. This information could be useful in screening or breeding new ALS-resistant almond varieties. If the interstock trial showed some positive results it may provide growers with new options of planting ALS-susceptible varities on resistant interstock trees for additional protection against ALS.

Table 1. Average pH values for all four almond cultivars.

pH values					
Cultivar	**Nov**	**Jan**	**Feb**	**March**	**July**
Butte	5.18	5.64	5.63	5.44	5.58
Carmel	5.41	5.54	5.60	5.88	5.18
Peerless	5.55	5.76	5.88	5.73	5.23
Sonora	5.30	5.68	5.81	6.07	5.46

Table 2. Average osmolarities for all four cultivars.

Osmolarity mmol/kg					
Cultivar	**Nov**	**Jan**	**Feb**	**March**	**July**
Butte	22.75	12.75	26.75	34.83	56.81
Carmel	16.00	25.34	38.42	34.67	50.92
Peerless	11.84	13.13	29.08	34.75	47.21
Sonora	19.00	13.92	19.00	31.96	50.58

Table 3. Averages of fructose, glucose, sucrose, calcium, magnesium, and iron for all four cultivars. Xylem sap extracted in November was only analyzed for Peerless and Carmel cultivars because too little sap was extracted from Sonora and Butte trees.

Fructose mg/L

Cultivar	Nov	Jan	Feb	March	July
Butte		362.50	264	331.00	565.75
Carmel	347	804.50	362	499.00	869.50
Peerless	348	455.50	365.67	407.75	616.50
Sonora		413	202.75	149.00	947.75

Glucose mg/L

Cultivar	Nov	Jan	Feb	March	July
Butte		500.50	374	484.50	688.75
Carmel	440	1062.50	617	720.25	1090.00
Peerless	428	526.00	638.00	526.00	723.75
Sonora		470	371.00	218.00	1058.50

Sucrose mg/L

Cultivar	Nov	Jan	Feb	March	July
Butte		54.50	39	<10	66.75
Carmel	301	147.00	38	28.50	148.75
Peerless	70	57.50	22.75	11	117.25
Sonora		<20	16.75	16	97.25

Calcium mg/L

Cultivar	Nov	Jan	Feb	March	July
Butte		42.55	67.43	35.80	27.85
Carmel	47.4	37.65	61.53	26.95	45.40
Peerless	26.8	34.30	67.88	27.43	42.33
Sonora		26.25	68.05	20.73	46.70

Magnesium mg/L

Cultivar	Nov	Jan	Feb	March	July
Butte		26.80	33.13	22.08	9.43
Carmel	29.1	24.40	28.28	15.65	18.78
Peerless	18.5	19.20	35.58	17.50	16.00
Sonora		20.30	40.23	14.63	22.85

Iron mg/L

Cultivar	Nov	Jan	Feb	March	July
Butte		0.15	0.25	0.16	0.25
Carmel	0.3	0.15	0.18	<0.2	0.25
Peerless	<0.2	0.25	0.15	0.15	0.23
Sonora		0.35	0.13	0.25	0.25

Table 4. Averages of total polyphenolic content of xylem sap measured in Gallic acid (GA) equivalents.

Polyphenols GA equivalents					
Cultivar	**Nov**	**Jan**	**Feb**	**March**	**July**
Butte	254.14	114.62	118.48	68.56	47.62
Carmel	186.39	153.89	105.83	69.08	107.14
Peerless	98.2	86.64	64.98	51.68	85.27
Sonora	-	90.50	56.62	55.94	141.00