Identifying Factors Mediating Resistance to Almond Leaf Scorch Disease

Project No.: **07-PATH8-Kirkpatrick**

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Interpretive Summary:

Almond Leaf Scorch (ALS) disease is caused by the bacterium *Xlyella fastidiosa* which lives exclusively in plant xylem vessels. Previous field observations on ALS noted striking differences in the 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 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. The pH and osmolarity measurements were taken and xylem sap osmolarity clearly increases in the spring and into summer in all cultivars, while pH values were similar over the 9 month period. Xylem sap was analyzed for soluble calcium, magnesium, iron and sugars, fructose, glucose, and sucrose. We also found that immediately after expression, xylem sap began to turn yellow indicating the presence of polyphenolics that we will measure with Folin-Ciocalteau reagent to compare the phenolic content of xylem sap from resistant and susceptible cultivars. Overall there were no consistent differences in pH, osomality, sugar and inorganic ion concentrations between ALS-susceptible and resistant almond cultivars.

Objectives:

1. Identify the biochemical and anatomical properties that eliminate Xf 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.

Materials and Methods:

Objective 1 - Four trees of each cultivar, Butte, Carmel, Peerless, and Sonora were chosen for xylem sap sampling. Xylem fluid was expressed from 1-7 cut almond branches per tree using our specially designed pressure chamber (PMS Instruments, OR). Xylem sap was collected in November, January, February, March, and July. The pH of each sample was measured and then samples were frozen at -20C. The osmolarity was measured with a vapor pressure osmometer (Wescor Inc., UT). Xylem sap samples were sent to the Davis ANR Analytical Lab where fructose, glucose, sucrose, calcium, magnesium, and iron were measured.

We are comparing anatomical structure of xylem elements at 2 times during the dormancy period, in January and early March. Tissue samples were taken from branches approximately 2.5 cm in diameter and from the trunk. These have been have been fixed in glutaraldehyde and are awaiting microscopy.

Xylem sap samples are being prepared for further analysis of biochemical properties including total phenolics, organic acids, and protein profiles. If unique proteins are noted they will be cut from the polyacrylamide gel and sequenced by the UCD Analytical Protein Laboratory to determine their identity.

Objective 2 - Almond trees with ALS resistant interstocks, Butte and Carmel, are on order from Fowler nurseries.

Results and Discussion:

Immediately following extraction, xylem sap samples turned brown indicating the presence of phenolic compounds. Given this result we will add an assay using the Folin-Ciocalteau reagent to measure total phenolic compounds. This is relevant because phenolic compounds are involved in plant defenses and some phenolics can inhibit bacterial growth. Full statistical analysis on pH, osmolarity and xylem sap components has not been completed. However, the data clearly show that osmolarity increases in the spring and into summer as average osmolarity was highest in July for all cultivars. Components of osmolarity such as calcium, magnesium, and sugars should also be higher. Overall there were no consistent differences in pH, osomality, sugar and inorganic ion concentrations between ALS-susceptible and resistant almond cultivars. This observation suggests that other host factors such as phenolics, proteins or anatomical responses are mediating the observed differences between ALS-resistant

and susceptible cultivars. These parameters are the focus of the second phase of this research project.

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

pH values	November	January	February	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	November	January	February	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**	**13.125**	**29.08**	**34.75**	**47.21**
Sonora	**19.00**	**13.915**	**19.00**	**31.96**	**50.58**

Table 3. Averages of fructose, glucose, sucrose, calcium, magnesium, and iron for all four cultivars. Too little sap was extracted from Sonora and Butte trees in November for analysis.

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

Recent Publications:

None