Almond Board of California Progress Report, 2005

I. Project Title: Epidemiology of Almond Leaf Scorch Disease in the San Joaquin Valley of California: Factors Affecting Pathogen Distribution and Movement.

II. Project No.: 05-RG-01

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V. Introduction:

California almond production experienced a near record harvest in 2005 with yields of approximately 984.1 M pounds. The industry continues to average above 20% annual growth and leads California in agricultural exports with further increases expected. Expansion of the industry in California's San Joaquin Valley (SJV), however, may be impacted by the recent emergence of almond leaf scorch (ALS) disease, caused by the bacterium *Xylella fastidiosa* (*Xf*). This disease poses a serious threat in many almond growing regions of California's Central Valley where ALS incidence has increased notably in the last 5 years.

The primary vector(s) of ALS strains of *Xf*, however, have not been well documented. Nevertheless, some sharpshooter and spittlebug species have been implicated as probable vectors of *Xf*-ALS strains. While *Xf* has long been present in the SJV, the incidence of ALS appears to have emerged, or reemerged, as a significant disease threat to numerous locations throughout much of almond producing region. The overall goal of this project is to increase our understanding of the epidemiology of ALS in the central and southern SJV of CA with a focus on factors that influence the geographical distribution and movement of the pathogen. A primary focus has been to accurately identify the natural insect vectors of *Xf*-ALS strains, to characterize temporal and spatial patterns of disease incidence within selected orchards, and to determine the genetic structure of *Xf* strains associated with ALS. An accurate knowledge of which vector species transmit *Xf*-ALS strains in the central and southern SJV, what strains/genotypes of the pathogen they acquire, where they acquire the pathogen, when they move into orchards, and when they spread the pathogen to almonds is critical to understanding and managing the spread of this disease.

VI. Objectives:

1. Identify the primary vectors of *Xylella fastidiosa* causing almond leaf scorch disease and determine their (a) seasonal population dynamics and (b) patterns of movement into and within selected orchards from the surrounding agricultural landscape.
2. Comparatively characterize *Xylella fastidiosa* populations associated with (a) almond leaf scorch disease, (b) insect vectors immigrating into almond orchards, and (c) identify potential reservoir hosts in and around almond orchards.
3. Monitor the progress of almond leaf scorch disease incidence within selected orchards to evaluate the extent of tree-to-tree spread of *Xf* that may occur among trees and identify whether affected trees can serve as inoculum sources.

VII. Results:

Objective 1. Seasonal population dynamics. The seasonal population dynamics of potential insect vectors were monitored over two complete seasons at monthly intervals from April 2004 through September 2005. Potential *Xf* vector species were collected from 3 separate habitats: 1) orchard trees, 2) vegetation on the orchard floor, and 3) within adjoining forage and pasture crops using sweep sampling. Sampling has been conducted within and surrounding 2 almond orchards in Fresno County and 1 almond orchard in Kern County each with a recent history of ALS. At all 3 sites in 2004, a total of 1,981 adult green sharpshooters (GSS: *Draeculacephala minerva*) were collected from monthly sweep samples, the majority (N=1,967) were swept from adjoining pastureland and alfalfa forage crops (Fig. 1). Less than 1% of the total GSS collected (N=14) were obtained from sweep samples of

vegetation on the orchard floor(s) and no adult GSS were collected from sweep samples of almond foliage. In 2005, significantly larger populations were collected among the 3 habitats totaling 25,113 adult GSS. Again, the largest number (N=25,096) of potential vectors was collected in irrigated pasture and forage alfalfa located adjacent to orchards with ALS and very few insects were recovered from vegetation on the orchard floor (N=17) or in almond foliage (N=0). Peak periods of GSS collection from irrigated forage habitats were during the interval June-August in 2004 and 2005 and varied significantly between years averaging 19.2 and 269.7 adult GSS / standard 50-sweep sample among the 3 locations sampled.

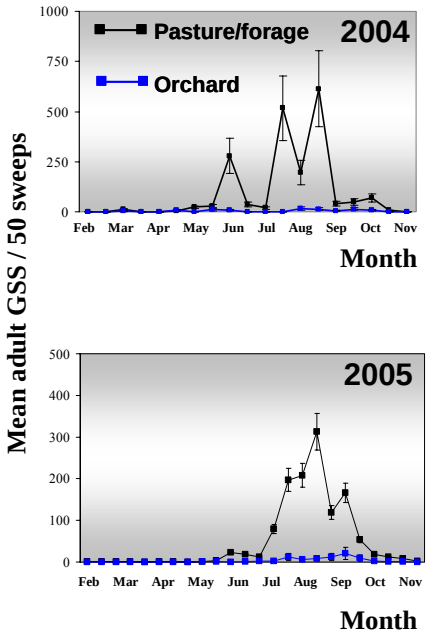

Figure 1. Mean number of adult GSS (D. minerva) per 50 sweep sample collected from forage pasture habitats compared with collections in the almond orchards

Seasonal patterns of dispersal. The seasonal dispersal patterns of potential *Xf* vectors dispersing into selected almond orchards have also been monitored over two complete seasons in 2004 & 2005. Throughout both seasons, migrating sharpshooter adults were monitored using combinations of yellow sticky traps and vertical mesh sticky traps systematically placed within and surrounding 5 pre-selected almond orchards; 2 in Fresno County and 1 in Kern County, each with a recent history of ALS. At each location, GSS constitute the vast majority of known *Xf* vector species captured on dispersal traps among the 5 survey locations. No redheaded sharpshooters (RHSS), *Xyphon fulgida*, have been collected dispersing at any of the locations in Fresno and Kern Counties. Summing across all experimental locations in 2004, yellow card traps captured the fewest dispersing GSS (N=23) compared to suspended mesh traps (N=330) and through September, 2005. Seasonal patterns of GSS capture on replicated, suspended mesh traps illustrate peak periods of adult movement consistent with the 3 known summer generations (Fig. 2). Specifically, a modest peak in adult capture occurred in early to mid May in 2004 with successively larger peaks following in early July and mid August consistent with the species' multivoltine phenology. Again in 2005, peaks in GSS dispersal occurred in later portions of May, July and August averaging 6.9, 8.2, and 13.4 adult GSS per mesh trap, respectively across the 3 survey locations in Fresno and Kern Counties.

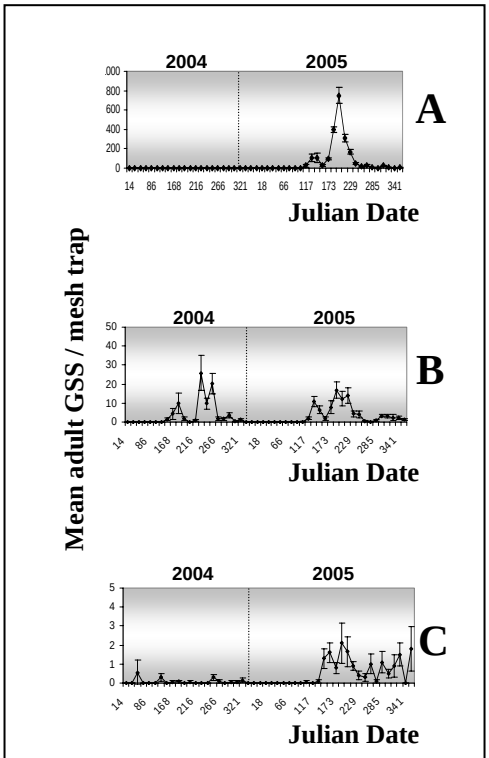

Figure 2. Mean (±SEM) number of adult GSS (*D. minerva*) per mesh trap collected in Fresno (A & B) and Kern (C) Counties.

Xf Detection. Genomic DNA of *Xf* has been amplified from adult GSS collected from permanent forage grass pasture and alfalfa forage crops. Following a 12 h lyophilization and a CTAB minipreparation DNA extract procedure, insect head capsules are removed and homogenized in sterile phosphate-buffered saline and the presence of *Xf* genotypes determined

using PCR formats. Among a total of 25,125 adult GSS collected in permanent pasture habitats adjacent to ALS-affected orchards in Fresno and Kern Counties, 1481 have been assayed for the presence of *Xf*. Averaging over all sample dates and locations, the seasonal incidence of infection in GSS was estimated to be 3.1% (Fig. 3). The incidence of *Xf* detected in adult GSS varied over time and among locations with the highest mean detection frequency recorded at a single Fresno County location (mean = 39.6%).

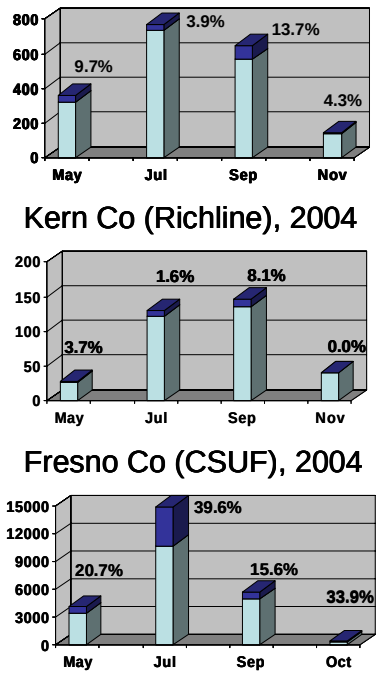

Figure 3. Estimated mean incidence of *Xf* detection in adult GSS (*D. minerva*) from the total collected in Fresno (A & B) and Kern (C) Counties, 2004.

Objective 2. The genetic diversity of *Xf* population(s) associated with ALS in central and southern portions of the SJV has been assessed to date using single nucleotide polymorphisms (SNPs) from genome loci of taxonomic importance deduced from the available genome sequences. There are clearly at least two genomic populations which co-exist simultaneously in affected orchards represented by grape (G-type) and almond (A-type) genotypes. The strongest evidence comes from a single orchard in a Kern County where 49 of 67 isolates were classified as A-type and the remainder (27%) classified as G-type. ALS epidemiology resulting from mixed strain populations had not been demonstrated previously and the relevance of these findings resulted in the manuscript by Chen et al. (2005), Two *Xylella fastidiosa* genotypes associated with almond leaf scorch disease on the same location in California, *Phytopathology* 95:708-714.

We have recently expanded our investigations of the genetic population structure of *Xf* among cultivars in the affected regions. Specifically, we have included conducted a simple sequence repeat (SSR) marker analyses developed by Dr. Hong Lin, USDA-ARS, Parlier, California. These primers are *Xf*-specific and useful for detecting polymorphism among and within crop-associated *Xf* strains. A total of 116 samples collected from ALS disease-affected almond in 2004 from an orchard on the campus of Fresno State University were recently analyzed and the haplotypes and allele frequencies within this population were recorded. Genetic distances among 116, ALS isolates collected from this CSUF campus location were estimated (Fig. 4). This hierarchical data set allows partitioning of the genetic differentiation among different categories including ALS severity, almond cultivar, and different orchards within specific regions. We plan to examine the proportion(s) of total genetic diversity explained by these variables both within and among survey locations which will aid in epidemiological and strain virulence studies. This marker system has been used to study the geographic population structures of grape *Xf* strains in California and has also been used as a tool to study interactions between *Vitis* and *Xf* in PD resistant and susceptible grapes.

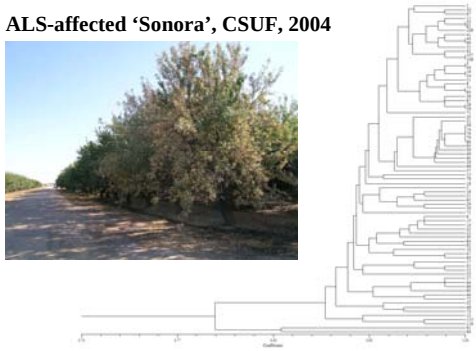

Figure 4. Cluster analyses using Nei's coefficient representing the genetic distances among 116 *Xf* isolates collected in an ALS-affected orchard,

Objective 3. Incidence of ALS was mapped in 5 selected almond orchards (3 orchards in Fresno and 2 orchards in Kern County) in 2003 and 2004 to assess/evaluate the relative importance of primary versus secondary patterns of *Xf* spread (Groves, et al, Plant Disease 59:581-589, 2005). Two-dimensional maps of ALS spatial distribution(s) were generated from each surveyed orchard and characterized by calculation of mean incidence of ALS-affected trees within and across rows. Disease incidence varied among almond cultivars in each orchard with the highest mean infection rates and most severe symptoms present in the cultivar 'Sonora'. Geo-statistical analyses were used to interpret the spatial patterns of ALS-affected trees. Spatial patterns of ALS were mapped in 2003 and 2004 at each of the survey orchards in Fresno and Kern Counties and data analysis for surveys conducted in 2005 have just concluded. Mapped ALS incidence over survey years 2003-04 have been analyzed using combinations of spatial analyses to characterize the temporal patterns of disease progress. ALS surveys indicate that clusters of diseased trees were regularly associated with field borders adjoining habitats known to support populations of potential vectors (Fig. 5). Primary spread of *Xf* from outside inoculum sources would lead initially to random patterns of infected plants, which may or may not be followed by tree-to-tree movement, or secondary spread of the pathogen, resulting in disease clusters or foci. Over multiple seasons, successive waves of primary spread may account for the spatial patterns of ALS observed in our study to date where clusters of infected trees were often associated with field borders (Fig. 5). Temporal analysis of these spatial data over successive seasons will be necessary to better understand the temporal patterns of ALS progress and the extent of secondary spread that may in fact occur.

The impact(s) of ALS on almond yield and quality was evaluated in Fresno and Kern County orchards in 2004 and 2005. Tree characteristics including diameter, height, yield, and nut quality were measured from a sub-sample of ALS-affected and asymptomatic almond. Trees were individually shaken, nuts swept, and collected for yield and quality assessments across three almond cultivars collected at 3 survey locations. Significant reductions in total yield, shelling percentage, and nuts/oz were observed in 2004 harvests (Table 1) with fewer reductions observed in 2005 (Table 2). In both years, however, numeric reductions in all yield and quality categories were observed between ALS-affected and asymptomatic, healthy tress. The impact of ALS on overall tree productivity coupled with spatial and temporal patterns of disease spread (primary vs. secondary), provides critical new information about the necessity for, or epidemiological importance of, rouging ALS-affected trees.

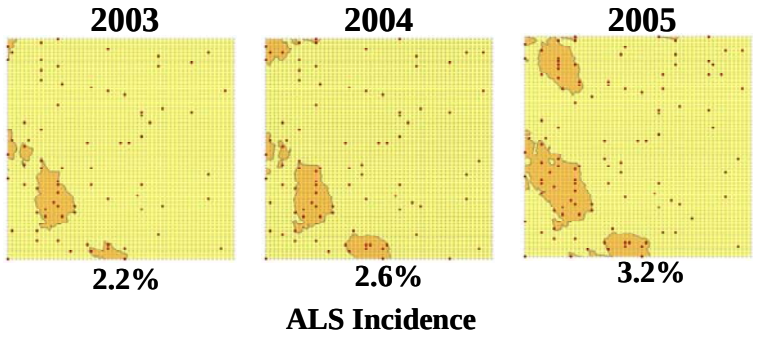

Figure 5. Kriged surfaces of disease incidence in 2003, 2004, and 2005 illustrating ALS aggregates adjacent to field boundaries in the Kern County almond orchard, Richline.

TABLE 1. 2004 yield summary for harvests of ALS-affected and asymptomatic almond.

County	Orchard	Cultivar	ALS Incid.	Treatment	Mean Kernal / oz[1]	Shelling Percent[1]	Total lbs/ Tree[1]
Fresno	Orch 1	Sonora	0.28	ALS	24.1 a	70.4 a	16.7 a
				Control[2]	18.4 a	75.1 a	33.7 b
	Orch 2	Sonora	0.11	ALS	25.7 a	69.2 a	9.6 a
				Control	24.1 b	68.4 a	21.8 b
		Carmel	0.06	ALS	32.2 a	41.5 a	7.3 a
				Control	26.6 a	46.9 a	13.8 a
Kern	Orch 3	Sonora	0.07	ALS	23.8 a	75.3 a	21.6 a
				Control	22.0 a	75.4 a	32.1 b
		Nonpareil	0.02	ALS	31.1 a	69.0 a	30.7 a
				Control	29.0 b	72.4 a	40.4 b

[1] Means not followed by the same letter in columns between treatments are significantly different (α=0.05) (Proc GLM: LSMEANS)

[2] Trees harvested as controls represent ALS-asymptomatic trees

TABLE 2. 2005 yield summary for harvests of ALS-affected and asymptomatic almond.

County	Orchard	Cultivar	ALS Incid.	Treatment	Mean Kernal / oz[1]	Shelling Percent[1]	Total lbs/ Tree[1]
Fresno	Orch 1	Sonora	0.27	ALS	18.3 a	73.3 a	7.0 a
				Control[2]	16.1 a	75.6 a	11.9 a
	Orch 2	Sonora	0.09	ALS	22.5 a	69.4 a	18.1 a
				Control	21.0 a	68.5 a	26.5 a
		Carmel	0.03	ALS	27.3 a	51.2 a	14.4 a
				Control	24.3 a	57.0 b	20.3 a
Kern	Orch 3	Sonora	0.05	ALS	20.6 a	76.2 a	27.2 a
				Control	19.8 a	75.8 a	40.9 b
		Nonpareil	0.02	ALS	24.9 a	68.2 a	34.7 a
				Control	25.4 a	69.6 a	40.4 a

[1] Means not followed by the same letter in columns between treatments are significantly different (α=0.05) (Proc GLM: LSMEANS)

[2] Trees harvested as controls represent ALS-asymptomatic trees

VIII. Publications:

Groves, R.L., and Chen, J. 2005. Epidemiology of Almond Leaf Scorch Disease in the central San Joaquin Valley of California, *In* Proceedings, 33rd Annual Almond Industry Conference, 7-8 December, Almond Board of California, Modesto, CA.

Groves, R.L., Cabrera, J.C., Chen, J., Viveros, M., Freeman, M., and Lynn-Patterson, K. 2005. Geo-spatial analysis of Almond Leaf Scorch Disease, *In* Proceedings, 11th Annual California GIS Conference, March 16-18, Bakersfield, CA.

Groves, R.L., Chen, J., Civerolo, E.L., Freeman, M.W., and Viveros, M.A. 2005. Spatial analysis of almond leaf scorch disease in the San Joaquin Valley of California: factors affecting pathogen distribution and spread. Plant Dis. 89:581-589.

Chen, J., Groves, R.L., Civerolo, E.L., Viveros, M.A., Freeman, M.W., and Zheng, Y. 2005. Two *Xylella fastidiosa* genotypes associated with almond leaf scorch disease on the same location in California. Phytopathology 95:708-714.

Chen, J., Groves, R.L., Zheng, Y., Civerolo, E.L., Viveros, M.A., and Freeman, M.W. 2006. Colony morphology of almond leaf scorch strains of *Xylella fastidiosa* and its epidemiological application. Euro. J. Plant Pathol. (accepted for publication, February 2006).

Chen, J. Groves, R.L., and Civerolo, E.L. 2006. Surface translocation of *Xylella fastidiosa* on solid medium surfaces. J. Bacteriology (submitted for publication, June 2006).

IX. Presentations:

Groves, R.L. 2005. *Seasonal population biology of Xylella fastidiosa and dispersal of associated insect vectors; factors affecting the spread of the almond leaf scorch pathogen.* Pacific Branch Meeting, Entomological Society of America, Pacific Grove, CA. Invited symposia speaker.

Groves, R.L., Cabrera, J.C., Chen, J., Viveros, M., Freeman, M., and Lynn-Patterson, K. 2005. *Geo-spatial analysis of Almond Leaf Scorch Disease*. 11th Annual California GIS Conference, Bakersfield, CA. Invited symposia speaker.

Groves, R.L., Chen, J., Viveros, M., Freeman, M., and Lynn-Patterson, K. 2005. *Application of spatial statistics to investigate pathogen distribution.* Copesan Technical Committee Conference, Parlier, CA. Invited speaker.

Groves, R.L. 2005. *Almond leaf scorch: Re-emerging threat or novel problem.* Kern County Fall Almond Meeting, Bakersfield, CA. Invited speaker.

Groves, R.L. and Chen, J. 2005. *Epidemiology of Pierce's Disease in the Central San Joaquin Valley of California.* Pierce's Disease Research Symposium, Crop Biology and Disease Epidemiology, San Diego, CA. Invited session speaker.

Groves, R.L., and Chen J. 2005. Temporal patterns of ALS disease progress: implications for management of the Pierce's disease problem in grapes. GWSS Workgroup Meeting, San Diego, CA. Invited speaker.

Groves, R.L. 2006. Primary inoculum sources of *Xylella fastidiosa* and their relationship to susceptible crops in a diverse landscape. Pacific Branch Meeting, Entomological Society of America, Wailea, Hawai'i. Invited symposia speaker.

VII. Research Summary:

This research has generated critical new information about the spatial population dynamics, the dispersal biology, and the seasonal occurrence of *X. fastidiosa* in dispersing insect vectors. Insect population dynamics studies continue to suggest the that native, green sharpshooter, *D. minerva*, is abundant in habitats adjacent to ALS-affected orchards, regularly disperses into these susceptible crops, and a significant proportion of dispersing individuals harbor *X. fastidiosa* within their mouthparts. Furthermore, this research has generated a more complete understanding of the genetic diversity of *X. fastidiosa* affecting almond and the spatial structure of ALS disease progress suggesting limited secondary spread of the bacterium within affected orchards. Specifically, two genotypes of *X. fastidiosa* simultaneously occurred in ALS-affected orchards and varied in their seasonal abundance between cultivars. Using both bacterial isolation on solid media and real-time PCR assays, a trend toward increasing bacterial populations occurred over the interval March through July and then slowly declined over the remainder of the season. Peak transmission efficiencies for GWSS (4.1%) and GSS (6.2%) in occurred in June and then declined rapidly with the onset of foliar scorching symptoms. A more complete understanding of the important epidemiological factors that influence *X. fastidiosa* persistence and spread among susceptible cultivars will result in improved disease management and the ability to effectively limit the spread of *Xf* induced diseases. These results will ultimately contribute towards the development of long-term, economically, and environmentally sustainable management solutions that will directly benefit agricultural producers, crop consultants, and other stakeholders.

VIII. Acknowledgements:

We wish to thank the Almond Board of California (Modesto, CA) for their support of this project and also the Kearney Agricultural Center's, Geographic Information Systems Facility for their assistance mapping disease distributions.