# Epidemiology of Almond Leaf Scorch Disease in the San Joaquin Valley of California: Factors Affecting Pathogen Distribution and Movement

**Project No.:** 06-PATH6-Groves

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## Interpretive Summary:

Insect sampling indicates that green sharpshooter is the most abundant vector in and around almond orchards in the Southern San Joaquin Valley. Green sharpshooter abundance was greater in samples collected from pastures and forage alfalfa crops adjacent to almond orchards than in orchards. Patterns of disease incidence suggest that most new infections occur due to movement of inoculative vectors into orchards from surrounding habitats and that tree-to-tree spread of *X. fastidiosa* is rare. Within orchards there were at least two genotypes of *X. fastidiosa,* the A- and G-types. Analysis of the *psp*B locus within each type indicates substantial variation within types. The importance of this variability on pathogencity is unknown at this time. Estimates of ALS on yield and tree mortality for the cultivars Sonora and Nonpareil indicated that yields of ALS-affected trees are indeed lower than those of unaffected trees. However, yields of ALS-affected trees did not decline over years and no infected trees died. Results suggests that green sharpshooter is the most common vector in the Southern San Joaquin Valley and the risk of increased ALS incidence is likely related to the abundance of suitable habitat for green sharpshooter surrounding an orchard. Tree-to-tree spread of *X. fastidiosa* appears to be rare, suggesting that removing infected trees to prevent secondary spread may not be needed. While yields of infected trees were lower than those of uninfected trees, returns from infected trees are likely to be higher than those from replacement trees.

## Objectives:

1. Identify the primary vectors of *Xf* causing almond leaf scorch disease and characterize (a) their seasonal abundance and (b) their patterns of movement into and within selected orchards from the surrounding agricultural landscape (c), and the detection of the *Xf* in a subsample of dispersing vectors.

2. Determine the comparative genetic structure of *Xylella fastidiosa* populations associated with (a) almond leaf scorch disease-affected almond located in (b) different orchards within California's Central Valley using (c) simple sequence repeat (SSR) marker analyses.

3. Monitor the spatial and temporal patterns of ALS disease progress within selected orchards to evaluate the extent of tree-to-tree spread of *Xf* that may occur and determine whether affected trees can serve as inoculum sources.

## Materials and Methods:

1. Identify the primary vectors of *Xf* causing almond leaf scorch disease and characterize (a) their seasonal abundance and (b) their patterns of movement into and within selected orchards from the surrounding agricultural landscape (c), and the detection of the *Xf* in a subsample of dispersing vectors.

   At monthly intervals from April 2004 through December 2005, populations of 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 standard, 50 - sweep sampling techniques. Sampling was conducted within and around 2 almond orchards in Fresno County and 1 almond orchard in Kern County.

   The seasonal population dynamics of potential *Xf* vectors dispersing into selected almond orchards was also monitored from January 04 to December 05 using yellow sticky traps, vertical mesh sticky traps, and black light traps systematically placed within and around 5 almond orchards; 3 in Fresno County and 2 in Kern County.

   Starting in 2006, we focused our sampling efforts on forage and pasture crops that were adjacent to almond orchards with a history of ALS. Standard sweep samples were collected monthly and sticky traps were monitored biweekly at 5 sites.

2. Determine the comparative genetic structure of *Xylella fastidiosa* populations associated with (a) almond leaf scorch disease-affected almond located in (b) different orchards within California's Central Valley using (c) simple sequence repeat (SSR) marker analyses.

   The genetic diversity of *Xf* population(s) associated with ALS in central and southern portions of the SJV was assessed using single nucleotide polymorphisms (SNPs) from genome loci of taxonomic importance. This analysis identified two genomic populations which co-exist in affected orchards, the G-type and A-type. The intra-genotype variation within these types was further analyzed by comparing varation in the *psp*B locus within each type.

   We also used SSR markers to examine the genetic structure of *Xf* populations in almonds. A total of 600 samples collected from two sites have been screened with 21 SSR markers. This data will be analyzed to determine the relationship between symptom severity, cultivar, and geographic location with *X. fastidiosa* genotype.

3. Monitor the spatial and temporal patterns of ALS disease progress within selected orchards to evaluate the extent of tree-to-tree spread that may occur and identify whether affected trees can serve as inoculum sources.

   Incidence of ALS was mapped in 4 selected almond orchards (2 orchards in Fresno and 2 orchards in Kern County) in 2003, 2004, and 2005 to determine the importance of primary versus secondary spread of *Xf*. In addition, the yield of ALS-affected and

unaffected almond trees was compared from 2004 to 2006 to determine the direct impact of ALS on almond yield.

## Results and Discussion:

1. Identify the primary vectors of *Xf* causing almond leaf scorch disease and characterize (a) their seasonal abundance and (b) their patterns of movement into and within selected orchards from the surrounding agricultural landscape (c), and the detection of the *Xf* in a subsample of dispersing vectors.

   In 2004, a total of 1,981 adult green sharpshooters (GSS: *Draeculacephala minerva*) were collected from monthly sweep samples, and the majority (1,967) were swept from adjoining pasture and alfalfa forage crops. Less than 1% of the total GSS collected (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 29,663 adult GSS. Again, the largest number (29,634) 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 (29) and none were collected in sweeps of almond foliage. The peak periods of GSS collection from irrigated forage habitats occurred during the interval June-August in both years.

   Seasonal patterns of GSS capture on suspended mesh traps illustrate peak periods of adult movement consistent with the 3 known summer generations. 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.

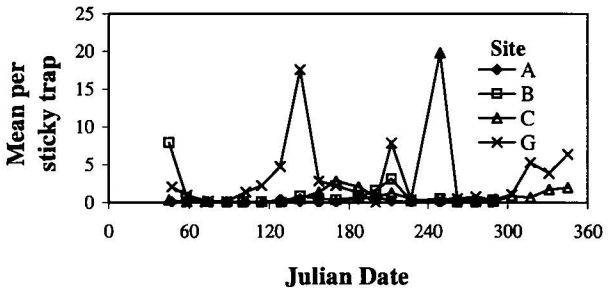

Fig. 1. Mean number of green sharpshooters per sticky trap in four alfalfa fields located in the San Joaquin Valley.

   During 2006, sampling of alfalfa forage crops adjacent to almond caught a total of 2,123 GSS on sticky traps and only 16 via sweepnet. Trap catches were generally higher on the edges of alfalfa fields than in the middle. Seasonal trends were similar to those observed in previous years (Fig 1).

2. Determine the comparative genetic structure of *Xylella fastidiosa* populations associated with (a) almond leaf scorch disease-affected almond located in (b) different orchards within California's Central Valley using (c) simple sequence repeat (SSR) marker analyses.

   Analysis of the *psp*B (PD1208) locus within the A- and G-types of almond leaf scorch disease revealed additional variation within each type (Fig. 2). Documentation of this variation may aid in more precisely describing each strain. Future tests will assess the role of variation in the *psps*B locus on pathogencitiy.

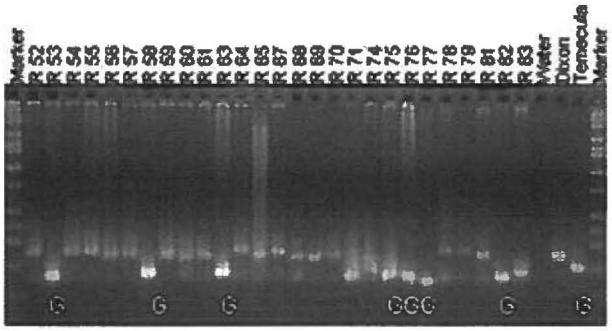


Fig. 2. DNA variation at the *psp*B locus in *X. fastidiosa* strains isolated from almond.

Screening of samples with SSR markers is complete. These data have yet to be analyzed.

3. Monitor the spatial and temporal patterns of ALS disease progress within selected orchards to evaluate the extent of tree-to-tree spread of *Xf* that may occur and determine whether affected trees can serve as inoculum sources.

Survey of ALS incidence at four almond orchards from 2003 to 2005 indicated that incidence varied among sites and cultivars. The highest incidence of ALS was observed in the cultivar Sonora. Analysis of the spatial distribution of ALS-affected trees revealed aggregations of ALS-affected trees in three of the four orchards. Disease clusters were often present in the outermost orchard rows and were often associated with field borders adjoining vector habitats. The spatial patterns of disease incidence observed in our study suggest that tree-to-tree spread is rare and that most new infections are the result of primary spread (i.e., movement of inoculative vectors into orchards from surrounding habitats).

In addition to monitoring the spread of ALS within orchards we also examined the effects of ALS on yield in the cultivars Sonora and Nonpareil. The cultivar Sonora was evaluated at three sites over three years and the cultivar Nonpareil was evaluated at two sites over two years. For cultivar Sonora, there was a significant effect of infection status (Fig. 3A-C) and orchard on yield in each year of study. For cultivar Nonpareil, infection status significantly reduced yield in both years of study (Fig. 3D). Averaged across sites and years, infected Sonora trees produced 40% fewer kilograms of kernel than uninfected trees (Fig. 3A-C). Averaged across years, infected Nonpareil trees produced 19% fewer kilograms of kernel than uninfected trees (Fig. 3D). For both cultivars, yields of infected trees did not decline

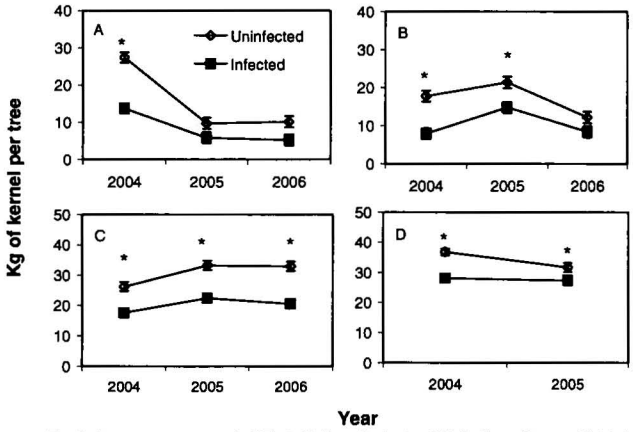


Fig. 3. Least square mean (± SE) yield of unaffected and ALS affected trees. Yield of Sonora trees at Orchards A (A), B (B), and C (C). Yield of Nonpareil trees at Orchard C (D). Within years and orchard, significant differences between uninfected and infected trees are indicated by an asterisk.

incrementally over years and trends in yield mirrored those of uninfected trees (Fig. 3). No ALS-affected Sonora or Nonpareil trees died during the study.

Kernels produced by ALS-affected Sonora trees were significantly lighter than kernels produced by unaffected Sonora trees in two of three years of study. Likewise, kernels produced by ALS-affected Nonpareil trees were significantly lighter in one of two years of study. Averaged across sites and years, kernels produced by infected Sonora trees were 7.6% lighter than kernels produced by uninfected Sonora trees. Averaged over years, weight of kernels from ALS-affected Nonpareil trees were 4.5% lighter than kernels from unaffected Nonpareil trees.

Table 1. Least square mean (± SE) weight of 25 kernels.

| | | Year | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2004[a] | | 2005[a] | | 2006[a] | |
| Cultivar | Measure | Uninfected | Infected | Uninfected | Infected | Uninfected | Infected |
| Sonora | Weight of 25 kernels[b] | **33.5 ± 0.6[a]** | **29.3 ± 0.6[b]** | **37.9 ± 0.4[a]** | **35.0 ± 0.4[b]** | 32.1 ± 0.5[a] | 31.3 ± 0.5[a] |
| Nonpareil | Weight of 25 kernels | **24.5 ± 0.3[a]** | **22.8 ± 0.3[b]** | 28.5 ± 0.5[a] | 27.9 ± 0.5[a] | --- | --- |

[a] Within years, comparisons between kernels collected from uninfected and infected trees which are significantly different are indicated by different letters and are bolded.
[b] Measured in grams.

## Recent Publications:

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

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