Occurrence of *Xylella fastidiosa* Strains Causing Almond Leaf Scorch from Neighboring Vegetation into Almonds

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Project Leader: Kent Daane, Dept. of ESPM, UC Berkeley

Project Cooperators: Elaine Shapland, Divi. Insect Biology, UC Berkeley; Alexander Purcell, Butte Co. UCCE; Roger Duncan, Stanislaus Co. UCCE; Joseph Connell, Dept. Plant Pathology, UC Davis; Bruce Kirkpatrick, Madera/Merced Co. UCCE; and Brent Holtz and Marshall Johnson, Dept. Entomology, UC Riverside.

The economic viability of California's vineyards has received considerable attention of late because of movement of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, into California. GWSS has quickly become California's most important vector of the bacterial pathogen, *Xylella fastidiosa*, which causes Pierce's disease (PD) of grapes. *Xylella fastidiosa* (*Xf*) is a xylem-limited bacterium that, in highly susceptible host plants, will clog the xylem and result in such severe water stress that the infected plant may die. Still, vineyards are not the only commodity that is either susceptible to *Xf* or facing increased disease incidence because of the arrival or spread of GWSS. *Xylella fastidiosa* is the causal agent of almond leaf scorch (ALS), alfalfa dwarf (AD), and phony peach, as well as leaf scorch in oleander, plum, elm, maple, oak and sycamore.

Because *Xf* is xylem limited, xylem sap-feeding insects such as sharpshooter leafhoppers (Cicadellidae: Cicadellinae: Proconiini) and spittlebugs (Cercopidae) are its important vectors. There has been a great deal of work compiled on vectors of PD in California and we now know that PD is associated with native sharpshooters: the green sharpshooter (*Draeculacephala minerva*), the red-headed sharpshooter (*Carneocephala fulgida*) and the blue-green sharpshooter (*Graphocephala atropunctata*). The vectors of ALS have not been as apparent or well-studied, but some leafhopper and spittlebug species have been implicated in ALS transmission. While *Xf* has long been present in the San Joaquin Valley, the spread and incidence of ALS was limited because breeding habitats of these California native insect vectors usually were not adjacent to or within almond orchards. Another important fact is that *Xf* has a very large host range, with over 145 natural or experimental host plants. The situation becomes a bit more complicated because not all *Xf* "strains" are the same – and for this reason, probably not all *Xf* will cause PD or ALS. The most important aspect of the *Xf* "family" for almond growers is that, of the tested strains, all caused ALS but only grape strains caused PD. What does that mean for the almond grower? It suggests that there may be many

hosts, including grape, that serve as a source for *Xf* strains causing ALS – but that these alternate hosts (e.g., hosts other than almond) will show no disease symptoms.

Here, we report on initial efforts to determine what factors might be responsible for the observed in crease in ALS by first characterizing the *Xf* "strains" (e.g., ALS strain, PD strain) found in almond leaf scorch samples and compare them with strains from nearby "alternate" host plants (e.g. grape, mustards, ornamental plum) and, second, collecting insects in ALS infected orchards and determine whether or not they carry *Xf.* These results should provide insight into what vectors have fed on Xf-infected plant hosts and which might be potential vectors of Xf in almond orchards.

This summer and fall, we established and sampled almond orchards for ALS research plots in Butte, Glenn, and Stanislaus counties. This winter, we will expand study sites to Merced, Fresno and Kern counties as well. At each site, suspect ALS infected trees are identified and leaf material sampled and processed to confirm presence of *Xf*. After a positive identification, we sampled insects monthly with yellow sticky cards, beat sampling of almond foliage, and sweep-net samples of almond foliage and ground covers. All insect material was taken to the laboratory and stored at -80°C. Currently we have over 2000 insects collected and stored, with an estimated 5% of this material a potential *Xf* vector (e.g., a sharpshooter) and even more a closely related insect species (e.g., a leafhopper).

We are currently sorting and identifying insect material for potential vectors. We have begun to process these samples for *Xf* using an immunocapture technique to pull out *Xylella fastidiosa* DNA from all the background material (e.g., insect DNA). This is followed by polymerase chain reaction (PCR) to amplify sections of the *Xf*-DNA in order to provide enough DNA material for *Xf* identification using gel electrophoresis. A positive find in field-collected insects will help identify potential vectors of ALS. Initial efforts indicate that our PCR techniques cannot provide better than 50% assurance that an insect which is actually "*Xf*-positive" will not be labeled as "*Xf*-negative." There are many potential factors that lower the PCR reliability, and for this reason, we are also investigating our ability to find *Xf* in insects that have been 1) collected on yellow sticky cards, 2) field-aged, or 3) subjected to different temperatures. We are also collaborating with other researchers to determine the best PCR techniques to isolate Xf in insects.

When potential insect vectors and alternate host plants are identified, we will complete Koch's postulates by infecting the alternative host plant with *Xf* in the laboratory, placing the potential vector on that plant for about 3 days, then transferring the potential vector to healthy almond plants which will be observed for development of ALS symptoms. These vector inoculations will be compared to inoculations with known vectors such as the blue-green sharpshooter and mechanical needle inoculations.