Project Name: Occurrence and movement of *Xylella fastidiosa* strains causing almond leaf scorch from neighboring vegetation into almonds

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RESEARCH OBJECTIVES:

1. Characterize the *X. fastidiosa* "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).

2. Collect insects in ALS-infected orchards and nearby vegetation, and the conduct laboratory analyses determine whether or not they carry *Xf.*

3. Conduct transmission experiments to determine vector efficiency.

4. Collect regional data on ALS epidemiology with respect to orchard management (e.g., irrigation practices) and the surrounding environmental conditions (e.g., nearby crop plantings).

5. Describe native sharpshooter biology.

6. Conduct experiments to reduce native sharpshooter density during winter and spring periods.

OVERVIEW

Our research in the 2004-05 crop seasons covered a wide range of almond leaf scorch (ALS) related studies. Most of the work concerned the establishment or movement of *Xylella fastidiosa* in or near almond orchards. *Xylella fastidiosa* is a xylem-limited bacterium that causes ALS, Pierce's disease (PD) of grapevines, and other diseases. We surveyed ground vegetation in ALS-infected almond orchards in California's Central Valley. Plant tissue samples were collected throughout a 2 year period and processed for *X. fastidiosa* presence using restriction enzyme digestion of RST31-RST33 polymerase chain reaction (PCR) products and bacterial culture on selective media. Overall disease incidence was low in the ground vegetation species, only 63 of 1369 samples tested positive. Of the 37 species of common ground vegetation tested, 11 tested positive for *X. fastidiosa*, including such common species as Sheperd's purse (*Capsella bursa-pastoris*), filaree (*Erodium* spp.), cheeseweed (*Malva parvifolia*), burclover (*Medicago polymorpha*), annual bluegrass (*Poa annua*) London rocket (*Sisymbrium irio*), chickweed (*Stellaria media*). There was a seasonal component to bacterial presence, with positive samples found only between

November and March. Both ground vegetation and almond trees were most commonly infected with the almond strain of *X. fastidiosa* (6 of 7 surveyed sites). ALS-infected almond samples had a *X. fastidiosa* concentration within reported ranges (1.84×10^6 - 2.15×10^7 CFU/g), however, we were unable to accurately measure *X. fastidiosa* titer in sampled ground vegetation for comparison. Using different sampling methods, we collected over 150,000 insects. Of these, ca. 42,000 were potential vectors (e.g., Hemiptera or true bugs) and there were 4,565 known vectors (e.g., green sharpshooters), mostly collected in sweep samples. The collected material is being processed for the presence or absence of *X. fastidiosa*. To date, we found only the green and redheaded sharpshooters were positive for *X. fastidiosa* (the three-cornered alfalfa hoppers collected have not been positive for *X. fastidiosa* in our samples, but see the report of R. Groves). These results are discussed with respect to ground vegetation management for ALS.

INTRODUCTION

Pierce's disease of grapes and almond leaf scorch (ALS) are incurable plant diseases that threaten the profitable production of these crops in California. Both diseases are caused by the xylem limited, nutritionally fastidious bacterium *Xylella fastidiosa* (Davis et al. 1978, Mircetich et al. 1976, Redak et al. 2004, Wells et al. 1987). Leaf damage occurs when the bacteria grow to such high concentrations that water and nutrient transport systems become occluded, leading to water stress in the leaves (Newman et al. 2003). Leaf and stem damage can progressively worsen until photosynthesis and nutrient production are impaired, thus lowering the quality and quantity of fruit produced and, eventually, killing susceptible grape or almond cultivars (Moller et al. 1974). *Xylella fastidiosa* is also the causal agent of diseases in other crops, including citrus variegated chlorosis, oleander leaf scorch, plum leaf scald, alfalfa dwarf, and phony peach (Chang et al. 1993, Purcell et al. 1999, Raju and wells 1986, Raju et al. 1980, 1981).

In California, much research has been conducted on the epidemiology of Pierce's disease (PD). Early surveys for the causal agent determined that the pathogen was spread by xylem-feeding sharpshooters, such as the bluegreen sharpshooter (*Graphocephala atropunctata*) (Freitag and Frazier 1949, Hewitt et al. 1949, Hill and Purcell 1995a). Subsequent studies confirmed that these and xylem-feeding hemipterans can acquire and transmit *X. fastidiosa* via their normal feeding on the xylem tissues (Hill and Purcell 1997, Purcell et al. 1979, Redak et al. 2004). More recent studies have focused on PD epidemiology as influenced by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Redak et al. 2004, Sorenson and Gill 1996). As important as these insect vectors is the plants that harbor *X. fastidiosa*. Surveys, in and near vineyards with PD in northern California and Florida, found numerous plants that can harbor *X. fastidiosa* in their tissues, without outward expression of susceptibility (30Nome et al. 1980; Costa et al. 2004, Hopkins and Alderz 1988). Similar surveys for the bacteria in plants growing near citrus groves with citrus variegated chlorosis have been conducted in Brazil (Lopes et al. 2003).

PD and ALS epidemiology requires more than the presence of susceptible crop varieties, insect vectors, and plant species that host *X. fastidiosa* and are suitable for feeding and/or breeding by these insect vectors. For example, after *X. fastidiosa* is acquired by a host plant, expression of disease symptoms depends on many factors including the plant's natural antibiotics (Purcell and Saunders 1999), temperature (Feil and Purcell 2001), season (Feil et al. 2003), the *X. fastidiosa* strain (Almeida and Purcell 2003a, Chen et al. 2005, Groves et al. 2005), and *X. fastidiosa* concentration (Almeida and Purcell 2003b, Hill and Purcell 1997). For this reason, a reservoir population of *X. fastidiosa* can reside in and around grape or almond orchards without the outward expression of plant disease. For disease epidemiology, these unseen reservoirs of the bacterial pathogen, in ground vegetation or cash crops, increases the likelihood that nearby susceptible crops will become infected. Removal of *X. fastidiosa* reservoir vegetation has, in fact, been an effective methods for controlling the spread of PD in California's coastal wine grape regions (Purcell and Saunders 1999). As yet, there have not been similar studies of vegetation management for controlling the spread of ALS, which has been increasing in prevalence and severity in California's interior valleys. Currently, there is a lack of information about the epidemiology of *X. fastidiosa* reservoir vegetation in and around almond orchards, and whether this system closely parallels or is different from that in grapes.

The purpose of this study was to survey vegetation in northern and central California almond orchards, which report increased incidence of ALS, in order to determine the possible reservoir hosts of *X. fastidiosa* At each survey site, we documented the ground vegetation species present throughout the season, determined the presence or absence, concentration, and strain of *X. fastidiosa* in the sampled ground vegetation, and recorded the disease incidence and *X. fastidiosa* strain in almond trees near harvest-time. By identifying the seasonal presence and incidence of *X. fastidiosa* in common ground vegetation in or near almond orchards, weed control efforts can appended to also reduce reservoir *X. fastidiosa* host species and reduce the level of bacterial inoculum.

MATERIALS AND METHODS

Ground vegetation survey. Sample sites were selected based on grower reports of ALS disease incidence. Surveyed orchards included sites in the north Central Valley (Butte County, Glenn County), the middle of the Central Valley (Stanislaus County) and the south Central Valley (Kern County). Every 2-Costa et al. 2004 weeks, depending on the seasonal availability of ground vegetation, a visual survey for the four most abundant weed species was conducted in each orchard. After the common species were determined, samplers transected the length of four evenly spaced rows (300 – 400 m per row) and, in each transect, collected specimens (3 – 5 leaves) of each weed species, which were stored separately for each species and transect in a 3.8 liter plastic bag. Typically, 10 – 30 individual plants were sampled for each weed species in each transect. The collected material was stored in a cooler (ca. 7°C) and transported to the laboratory, where samples were processed within 2 days of field collection.

Bacteria presence. Each sample (plant species and orchard row) was processed separately for the presence of *X. fastidiosa*. We selected plant parts where sharpshooters are known to feed, such as the leaf petioles, succulent base of grasses, and plant stems (Freitag 1951). Subsamples of these plant sections, from each sample, were removed with a sterilized razor, as described by Hill and Purcell (1997). The samples were assayed for the presence of *X. fastidiosa* using immunocapture DNA separation and PCR amplification with primers RST31-RST33, using a procedure developed by Hendson et. al (2001).

For PCR amplification, all samples were prepared in sterile 0.Costa et al. 2004 mL micro centrifuge tubes with 12.5µL Taq master mix (Qiagen), Costa et al. 2004.5µL PCR water, 1µL of each primer RST-31 and RST-33, and 4µL of DNA extract. PCR reactions were carried out in a Thermal Cycler according to the conditions described by Minsavage, et al. (1994). PCR products were subjected to 1.5% agarose gel electrophoresis, stained with ethidium bromide and viewed under ultraviolet light. The presence of *X. fastidiosa* in the original sample was determined by a band at 733 kb.

Bacteria strain. After gel electrophoresis, a preliminary strain difference analysis was carried out according to Minsavage, et al. (1994). The PCR product from all positive samples was subjected to restriction enzyme digestion with *Rsa*1 (10 µL PCR product, 0.2 µL *Rsa*1, 0.2 µL BSA, 2.0 µL Buffer C, and water) at 37°C for 2 hours. The RST31-RST33 PCR products from *X. fastidiosa* strains of oak, oleander, peach, plum, and all but three ALS strains are cleaved into two fragments, about 233 bp and 500 bp, while the PCR products from all PD strains as well as the ALS strains ALS1, Manteca, and Tulare are not digested by *Rsa*1 (Henderson et al. 2001).

Bacteria titer and incidence. Attempts were made to culture *X. fastidiosa* from all weed samples collected as well as from symptomatic almond trees in each orchard. For weed species, location and sample date shown to be positive for the presence of *X. fastidiosa*, using immunocapture DNA separation and PCR amplification, fresh samples were collected. Similarly, near harvest-time (September) we surveyed each orchard for trees showing classical symptoms of ALS (Mircetich et al. 1976) and collected symptomatic almond leaf and petiole samples. Both ground vegetation and almond samples were processed for bacterial culture on selective media within 24 hours.. We used a series of culture medias to provide a rudimentary indication of *X. fastidiosa* strain. Grape strains of *X. fastidiosa* grow on both PWG

(Hill and Purcell 1995b) and PD3 (Davis et al. 1980), but almond strains grow only on PWG (Almeida and Purcell 2003a). Samples were prepared for culture according to procedures described by Hill and Purcell (1995b, 1997). In this way we could determine both the strain and titer of bacteria in the tree.

Insect survey. Insects were sampled using a combination of techniques – although by the 2005 we used only sweep net samples as these proved to be the most efficient. In 2003-04, yellow sticky cards were hung in almond trees, for a period of 2-3 weeks. Ground covers and nearby weedy vegetation were sampled with a sweep net (typically (100-200 sweeps per field). The almond canopy was initially sampled with a beating tray (typically 200 beats per orchard). Samples were taken every 3-Costa et al. 2004 weeks, depending on the seasonal activity. In the laboratory, the insect samples were sorted, identified, and then stored in either acetone or frozen (-80°C) until they are processed for *X. fastidiosa* presence or absence.

DNeasy-kit (Qiagen) process for insect samples. To determine the presence of *Xf* in the collected insects, the heads and mouthparts of potential vectors are placed into the wells of an Elisa plate containing cell lysis buffer. The plate is then placed into a vacuum chamber to flush the lytic buffer through the mouthparts of the insect, which will lyse the *X. fastidiosa* cells in the mouthparts. The buffer and lysed *X. fastidiosa* cells were sterilized in a microcentrifuge tube with Proteinase K and Buffer AL, and then vortexed and incubated. After which, ethanol was added and the solution was centrifuged in a spin column containing a selectively permeable membrane. This process was repeated three times with a buffer and the resultant solution placed over a sterile microcentrifuge tube, incubated, and centrifuged. This last step is repeated for a total of 200 µl of isolated DNA in solution ready for PCR.

Almond and weed sampled for *X. fastidiosa* strains. Petioles or midribs will be separated from infected cuttings and weighed. The material will be surface sterilized, chopped, homogenized, diluted and then plated (20 μl drops) on the selected PWG (periwinkle-wilt Gelrite) and PD3 solid media (as described in Adlerz and Hopkins 1979, Purcell and Sanders 1999, Almeida and Purcell 2003b) and maintained in an incubator at 28ºC in the dark. *Xylella* can be detected in 4-5 days for Pierce's disease strains and 7-10 for almond leaf scorch strains. All *Xf* strains will grow on PWG, while not all almond strains will grow on PD3, which provides an initial identification of the *Xf* strain present.

Sharpshooter biology. We have begun studies to measure green sharpshooter (GSS) (*Draeculacephala minerva*) survival and development on weed species known to be hosts of *X. fastidiosa* in the field. Our two primary objectives are to measure GSS nymph development time on four weed species, and to measure GSS survivorship (nymph and adult) and egglaying on four weed species. The weeds species selected are: Bermudagrass (*Cynodon dactylon*), burclover (*Medicago polymorpha*), filaree (*Erodium cicutarium*), and cheeseweed (*Malva parviflora*). For each trial 10 GWSS adults (5 males and 5 females) were placed on caged weeds for a 48-hour egg laying period on equal number of plants of each species. Thereafter, we measured GSS nymph emergence weekly. After which, the resulting nymphs were collected (ca. 10 insects weekly from each plant) and their development stage(s) was determined. All insects are returned to the respective plants after each count. As needed, insects have been moved to a new plant of the same species when plant becomes senescent.

A second experiment is underway to determine GSS survivorship on various weed species. For each trial, ca. 15 first instar GSS were placed on plants and we then followed developed until the adult stage, recording the survivorship and development rate. The resulting adults are placed on similar weed species and the number of eggs deposited are determined.

Weed to almond occurrence and movement of *X. fastidiosa* strains. We have begun studies to complete Koch's Postulates for *X. fastidiosa* and sharpshooter vectors in previously-identified weed species.

In the first test we looked at the ability of GSS to move the pathogen from weeds to almonds. Nine species of weeds were selected for testing based on data from Shapland et al (2006). Weeds were started

from seeds ca. 8 weeks before inoculation. Plants were grown in Supersoil planting mix, with natural lighting conditions, in a 72F (+10/-5F) greenhouse. The weed species tested are: Shepherd's Purse (*Capsella bursa-pastoris*), filaree sp. (*Erodium* sp.), cheeseweed (*Malva parvifolia*), burclover (*Medicago polymorpha*), common groundsel (*Senecio vulgaris*), London rocket (*Sysmbrium irio*), sowthistle (*Sonchus* sp.), chickweed (*Stellaria media*), and burning nettle (*Urtica urens*). Infected "source plants", almonds infected with *X. fastidiosa* for experiments were generated by inoculating almond plants (seedlings or cuttings) with Dixon and Fresno-ALS strains of *X. fastidiosa* 2 to 3 months prior to transmission test, as described by Hill 1995. Trees were kept in unsprayed greenhouse until symptoms developed. Infected "source" plants were tested for *X. fastidiosa* presence and titer via culture 2 weeks prior to use in transmission experiments. GSS free from *X. fastidiosa* were reared on Bermudagrass via 3 successive plant changes as nymphs (every 7 days). Bermudagrass plants were started from cuttings in Supersoil, grown as other plants (previously described). GSS were collected from weeds (grasses and *Ludwigia* sp.) in swampy conditions at Wohler Bridge, Sonoma Co., or Pena Adobe Park, Fairfield, CA, or in almond orchards in Stanislaus and Butte Co. (May-July). Between 80 and 100 GSS were placed on *Xf*-positive "source" almonds for a 4-day "Acquisition Access Period" (hereafter called AAP). Transfer GSS in groups for 4 to plants to be tested for a 4-day "Inoculation Access Period" (IAP); confine insects to plants in small foam or mesh cages. Insects were transferred to additional plants for successive IAP's. Following inoculation, inoculation site was marked with lab tape and plants are placed in the greenhouse to develop infections. Six weeks after inoculation, alternate hosts wre assessed for *X. fastidiosa* presence and population via culture on PWG media (or immunocapture PCR as needed).

In the second test, we looked at the ability of *X. fastidiosa* to survive in alternate hosts. Field-grown alternate host plants, 20 per species, were assessed for *X. fastidiosa* presence 4, Costa et al. 2004 and 8 weeks after mechanical inoculation. Plants were mechanically inoculated according to Hill 1995, and planted outside in Parlier, CA or Bakersfield, CA. Weeds were tested for *X. fastidiosa* presence via culture and immunocapture PCR as described in Shapland et al 2006.

In the third test, we investigate *X. fastidiosa* acquisition from alternate hosts. GSS free of *X. fastidiosa* are obtained and reared as previously described. Four alternate host species, frequently found in almond orchards when GSS are present (filaree sp., cheeseweed, and burclover) grown from seeds, and Bermudagrass grown from cuttings as described above. When weeds are Costa et al. 2004 weeks old, they were inoculated with Dixon and Fresno-ALS strains of *X. fastidiosa* Costa et al. 2004 weeks prior to transmission test. GSS are placed in groups of 12 on *X. fastidiosa*-infected alternate hosts for a 4-day AAP. Then place insects in groups of four on almond seedlings for a 4-day IAP. At end of IAP, preserve insect heads in acetone and perform PCR to detect XF presence in SS.

Dormant insecticide trial. Working with a collaborating grower, we used an insecticide application (a pyrethroid, Pounce) to kill sharpshooters present during the winter and spring period. The experimental design was a replicated randomized split plot in each almond orchard, and each plot a minimum of 3 acres. Sharpshooter populations were monitored, as described previously. Incidence of ALS was compared with a complete orchard survey in September. This experiment will help determine if winter dormant insecticide applications, used to control other insect pests, may impact levels of sharpshooter populations in the spring and summer. We note here that as incidence of ALS may be very low and variable between seasons, this experiment may require 2-3 years of continual treatment before any impacts become evident.

Data analysis. Results for *X. fastidiosa* presence in vegetative ground cover species are presented as a qualitative "positive" or "negative" to express the potential role of these plant species as alternative hosts. Average bacterial concentration in media cultures is presented as CFU/g, with average concentrations among vineyards compared using Analysis of Variance, and means separated using Tukey's HSD comparison. Linear regression was used to compare the percentage positive *X. fastidiosa* in ground vegetation species with sample size for each plant species.

RESULTS

Ground vegetation Survey. From June 2003 to April 2005, 58 collection trips were made, about 10 trips to each of the six sampled orchards. There were 37 species of ground vegetation commonly found (Table 1), with most material collected in winter and spring when ground vegetation was common. Between August and October, it was difficult to find live ground vegetation within the almond orchards, a result of almond management practices for applied water and harvest operations. During this period, fewer weed species were available to sample and we often pooled samples across the orchard rows transected to produce a single sample for each plant species and orchard.

Bacteria presence. We processed 1369 samples from the six orchards, from which 63 samples were positive for *X. fastidiosa* (4.6%). Of the 37 species of common ground vegetation, we recovered *X. fastidiosa* from 11 species, including 5 species from which it had not previously been recovered in the field (Table 1). There was a strong seasonal component to bacterial presence in ground vegetation, with no *X. fastidiosa* positive samples found between April and mid-October during the two years of the study. Standard orchard management practices require the ground under the almond trees to be completely free of vegetation prior to harvest in August and applied irrigation is also discontinued, so that the nuts can be shaken from the trees and dried on the bare ground. Because all orchards followed these practices, there was little or no vegetation to be sampled from August to October (Figure 1, data from both years are combined).

Bacteria presence and strain. Results from both restriction enzyme digestion of RST31-RST33 PCR products with *Rsa*1 and bacterial culture on selective media showed that almond trees in Costa et al. 2004 of 7 experimental orchards were infected with the almond strain of *X. fastidiosa*. At one site (Zeering Rd in Stanislaus County), a grape strain of *X. fastidiosa* was isolated from all weeds and almond trees sampled (Table 2). At each site, tissue samples from both almond trees and surrounding weeds gave the same result: each contained either grape or almond strain of *X. fastidiosa*, but never both.

Bacteria titer and incidence. Petioles from ALS infected almond trees at Zeering Rd containing the grape strain of *X. fastidiosa* had an average concentration of 2.15×10^6 CFU/g, which is significantly greater than the concentrations at other sites sampled ($P = 0.014$). Our results agree with previous findings that the average *X. fastidiosa* titer in ALS-symptomatic almond leaves (Almeida and Purcell 2003b) is lower than the average *X. fastidiosa* titer in PD symptomatic grapes (Hill and Purcell 1997). The goal of assessing the titer of *X. fastidiosa* in weeds was met with much frustration, as every sample was contaminated with other bacteria before *X. fastidiosa* presence could be determined (results not shown). Previous researchers have also encountered this difficulty when attempting to culture such a slow growing bacteria from field samples (Lopes et al. 2003, Wistrom and Purcell 2005).

Insect survey. In 2003 and 2004, 490 yellow sticky cards were deployed, collected and the possible vectors identified (Fig. 2). There were 4,083 "potential" vectors (e.g., Cicadellidae [leafhoppers], Fulgoridae [planthoppers], Cercopidae [spittlebugs], Membracidae [treehoppers]). Of these, only 19 (0.46%) were know vectors (e.g., sharpshooters, spittlebugs). From August 2003 to October 2005, there were 41,495 potential vectors collected from sweep samples (from >150,000 insects) insect (Fig. 3). Of these, 4,565 were known vectors; the ease with which insects and known *X. fastidiosa* vectors were collected using the sweep net as compared with the stick cards or beat samples (data not shown) led us to rely solely on sweep samples in 2005. Of the known vectors, the GSS was the most common (>90%). We note that we caught no GWSS in the almond fields or adjoining vegetation, few red-headed sharpshooter (*Carneocephala fulgida*) and, at a few sites, the three-cornered alfalfa hopper (*Spissistilus festinus*) was common (typically when the almond field abutted an alfalfa field). For both sticky cards and sweep net samples, the period from May to July was the most active for the numbers of insects collected. There was little ground vegetation found in the orchard after preparation for harvest (August) and samples were discontinued.

The collected material is being processed for the presence or absence of *X. fastidiosa*. We found only the green and redheaded sharpshooters were positive for *X. fastidiosa* (to date, the three-cornered alfalfa hoppers collected have not been positive in our samples, but see the report of Russell Groves).

Sharpshooter biology. In winter 2005-2006, we established populations of all weed species in potted plants. In summer 2006, we established a laboratory colony of GSS. Studies on GSS biology and development began in summer 2006 and preliminary results (these studies are ongoing) showed that GSS can develop, from egg to adult, on all weed species tested (Bermudagrass, burclover, filaree, and cheeseweed). Development (egg to adult) has been surprisingly rapid, typically <8 weeks under glasshouse conditions.

Sharpshooter vector efficiency. Studies to test Koch's postulates began in summer 2006. We have tested only a few insects on each weed species. Initial problems have been high mortality of GSS on almond plants once they were transferred from the wee to the almond. These studies will continue in 2007 with changes to the methodologies to improve GSS survival.

Dormant insecticide trial. Data have not yet been analyzed from the sweep net collections of insects. There were no differences in ALS incidence between treatments (dormant insecticide versus no insecticide), although differences in ALS incidence are not expected to appear for 2-3 years after treatment initiation.

DISCUSSION

All previous field surveys for *X. fastidiosa* in alternate host plants have focused on PD management. With the recent increase of ALS in California, there was an even greater need to survey plants in almond orchards for *X. fastidiosa*. We showed the presence of *X. fastidiosa* grape and almond strains, which are the causal agents for ALS, were present in 29.7% of the common ground vegetation species sampled. Numerous studies have documented the survival of *X. fastidiosa* in different plant species (Almeida et al. 2001, Purcell and Saunders 1999, Wistrom and Purcell 2005). However, fewer have included field surveys (but see Costa et al. 2004, Hopkins and Alderz 1988, Raju et al. 1983), and this is the first reported survey for the season-long incidence of *X. fastidiosa* in non-symptomatic host plants in almond orchards. In comparison, one of the first and more extensive plant surveys as yet undertaken, looked at the ability of insect vectors to transfer *X. fastidiosa* (at the time unknown) from PD-infected grapes to suspected host plants to clean grapes (Freitag 1951). Despite the broad range of host plants included in that survey, conclusions about disease transmission were made based solely on symptom development in the clean grape plant. Subsequent studies have shown that plants can harbor *X. fastidiosa* at concentrations high enough for insect acquisition and transmission, but without exhibiting symptoms of disease (Purcell and Saunders 1999). In addition, the symptoms of PD and alfalfa dwarf that Freitag (1951) used as diagnostics can be misidentified with water or nutrient stress (Mircetich et al. 1976).

We used the more sensitive detection method of immunocapture DNA separation followed by PCR to survey for *X. fastidiosa* in vegetation in almond orchards for two years. In another recent field survey, riparian plants in the Napa River Valley were processed for *X. fastidiosa*, using ELISA as a detection method, and found a number of perennial trees and shrubs, as well as ground covers, were positive for the bacterium (Raju et al. 1983). Costa et al. (Costa et al. 2004) also used ELISA as a detection method, due to its low cost and lower time investment than PCR, to screen large numbers of potential host plants for *X. fastidiosa* in vineyard ground covers. While immunocapture DNA separation and PCR are more sensitive detection methods for finding *X. fastidiosa* in plant material than is ELISA (Minsavage et al. 1994), all established modern DNA detection methods are reliable for finding *X. fastidiosa* in plant material. Results from our survey found *X. fastidiosa* in many of the same ground cover species as the Costa et al. (Costa et al. 2004) field survey and Wistrom and Purcell (2005) glasshouse study (Table 1).

The sampled habitat in our study (almond fields in the Central Valley) provided clear differences from the earlier surveys (vineyards and riparian areas). Similarities between almond and grape crops include winter dormancy, late summer harvest, and overlap in growing regions. However, differences in the management of these crops result in very different ground management, which could result in different patterns of disease spread. For example, the rows between grapevines are often seeded with annual or perennial ground covers to improve the quality of the soil, or to serve as a trap crop for insect pests. This vegetation is left to grow the whole year, with only occasional mowing required for worker mobility. In contrast, in mid-summer the almond floor is striped of all vegetation in preparation for the August harvest. The trees are shaken to drop the nuts onto the ground, where they dry in the sun for 2 weeks before being collected with a vacuum. This practice is reflected in our survey results, which are based on vegetation collected during all months except for August, when no vegetation was available to be collected. Other differences arise from the shape and size of the crop plants. Grapes are planted in discrete rows, where adjacent vines often intertwine. Insects of all types can easily travel from one plant to anther, which may account for much of the observed vine to vine spread in insect vectored diseases of grape. Almonds are planted in a grid pattern, with trunks separated by 3 to Costa et al. 2004 meters. If adjacent plants do touch, it is in the canopy, high off the ground and less accessible to any insect vectors feeding on ground vegetation. This arrangement could be one explanation of the lack of obvious tree-to-tree spread that we observed in the surveyed almond orchards.

An interesting result is that of the *X. fastidiosa* positive plant species in our survey, 9 of the 11 were present in the orchards on most of the sampling dates and thus comprised the largest sample sizes of all ground vegetation species. There was a positive and significant relationship between the number of samples taken per plant species and the percentage of samples positive for *X. fastidiosa* ($y = 0.0553x - 0.2074$, $r^2 = 0.8935$). One explanation is that insect vectors that prefer the more commonly found plant species have moved the bacterium amongst these plants. In contrast, insect vectors that prefer the less common ground vegetation species, at least in our sampled orchards, were not attracted to these sites and, therefore, did not move the bacterium among plants. For example, some of the less common plant species in the sampled orchards, were common hosts of *X. fastidiosa* in other surveys, but were negative in our two year survey (Table 1). Thus the feeding behavior of these insects could be a more important factor in controlling the spread of ALS. We are currently conducting transmission tests in the greenhouse, using insects found via sweep net surveys in these same orchards.

Perhaps most important for the almond-vector-pathogen epidemiological relationship for ALS and resident ground vegetation is that we detected *X. fastidiosa* in weeds only during the cooler months, between October and April. This is in contrast to most previous field surveys that were conducted primarily during the growing season and in which *X. fastidiosa* was detected during the warmer summer months (Costa et al. 2004, Freitag 1951, Lopes et al. 2003, Wistrom and Purcell 2005). Seasonality and temperature is important for ALS or PD epidemiology as *X. fastidiosa* survives best in the plants at a moderate temperature (Feil and Purcell 2001) and plants inoculated on leaf tissue late in the growing season may not develop chronic disease symptoms (Feil et al. 2003). We hypothesize that the ground vegetation in the surveyed orchards best harbored *X. fastidiosa* at a temperature that was most consistent during the winter months, and when these fall/winter ground covers were newly formed and in good condition. During the late spring and summer months, most ground vegetation in the almond orchard, in contrast to seeded cover crops in vineyards, were small and in poor condition. These results suggest further investigation of the seasonal presence and concentration of *X. fastidiosa* in ground covers with the seasonal presence and abundance of potential insect vectors. Unlike in vineyards where a clear edge effect has been found with PD incidence (Purcell and Saunders 1999), most previous work has not revealed any clear spatial patterns with ALS (Purcell 1980, but see Groves et al. 2005).

We found the almond strain of *X. fastidiosa* was most common in the surveyed ALS-infected orchards. Recent studies on the biology of different strains of *X. fastidiosa* have shown varying abilities to infect different hosts: grape strains will cause disease symptoms in almond, grape (Almeida and Purcell 2003a), and oleander (Purcell et al. 1999), but neither almond strains (Almeida and Purcell 2003a, b) nor oleander strains (Purcell et al. 1999) will cause disease in grape. A recent study near Fresno, California, showed

that characteristics of different varietals of almonds as well as strain type (almond or grape) result in differing severity of ALS (Groves et al. 2005). A parallel study found both the almond and grape genotypes of *X. fastidiosa* in the same plant, pointing out the presence of a less virulent strain does not preclude the existence of a more virulent strain (Chen et al. 2005). We found the highest concentration of *X. fastidiosa* in almond petioles containing the grape strain, with the average titer significantly larger than at other sites ($p<0.014$). This is consistent with previous findings that grape strain can colonize almond petioles to a higher extent than can *X. fastidiosa* of the almond strain.

The survey results showed that common ground vegetation can harbor *X. fastidiosa* on the almond floor. Together these results suggest that perhaps a year-long weed management strategy over the whole orchard would be an important component, along with rouging infected trees or tree limbs, for management of *X. fastidiosa*.

Work with the insect survey found the "usual" suspects of probable insect vectors. The green sharpshooter was the predominant known vector and was the only vector that consistently tested positive for the presence of *X. fastidiosa*.. At one site in Stanislaus County and a few sites in Kern County, we found the three-cornered alfalfa hopper was very common. However, none of the tested specimens (from field collected samples) were positive for *X. fastidiosa*. In contrast, Dr. Groves collected this membracid in Fresno County, from alfalfa fields, and found a very high percentage of the specimens were positive for *X. fastidiosa*.. The difference between these tests is probably from insects' host plants where they were collected – the alfalfa versus weeds in the almond orchards. At the Fresno County sites the alfalfa fields were relatively older fields where tested plant samples were often positive for the presence of *X. fastidiosa*..

Work on GSS biology is still quite preliminary; however, indications are that this insect can survive and develop on all weed species tested. This suggest that the ground vegetation can harbor a vector population during the periods when the almond trees are not a good host (fall and winter). We were also surprised at how quickly the GSS developed from egg to adult under glasshouse conditions. These results imply multiple generations (3-5 per season) of the vector might develop on a series of weeds species in and near the orchard.

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Table 1: Weeds and alternate hosts collected from almond orchards and processed for the presence of *Xylella fastidiosa* using immunocapture DNA extraction and PCR.

Scientific Name	Common Name	*X.fastidosa* This study	*X.fastidosa* Previous studies	Reference
Amaranthus spp.	Pigweed	-	-	Costa et al. 2004
			+	Wistrom and Purcell 2005*
Amsinckia spp.	Fiddleneck	-	-	Costa et al. 2004
Anagallis arvensis	Scarlet pimpernel	-	-	Costa et al. 2004
Avena fatua	Wild oat	-	+	Freitag 1951
Brassicaceae spp.	Mustards	-	+	Costa et al. 2004
Capsella bursa-pastoris	Shepherd's purse	+	None	
Chamaesyce maculate	Spotted spurge	-	None	
Chenopodium album	Lambsquarter	-	None	
Claytonia perfoliata	Miner's lettuce	-	+	Raju et al. 1980, 1983
Conyza bonariensis	Fleabane	-	None	
Conyza canadiensus	Horseweed	-	+	Wistrom and Purcell 2005*
Coronopus didymus	Lesser swinecress	-	None	
Cyperus esculentus	Yellow nutsedge	-	+	Freitag 1951
Echinochloa crus-galli	Barnyard grass	-	+	
			+	Wistrom and Purcell 2005*
			+	Freitag 1951*
Erodium spp.	Filaree	+	-	Costa et al. 2004
			+	Wistrom and Purcell 2005*
Escallonia montevidensis	Escallonia	-	+	Freitag 1951
Festuca spp.	Fescue grass	-	None	
Helianthus spp.	Sunflower	-	+	Costa et al. 2004*
Hordeum murinium	Hare barley	-	+	Freitag 1951
Lactuca serriola	Prickly lettuce	-	-	Raju et al. 1980, 1983
			+	Wistrom and Purcell 2005*
Malva parvifolia	Cheeseweed	+	-	Freitag 1951*
			-	Costa et al. 2004
Medicago polymorpha	Burclover	+	+	Freitag 1951

Poa annua	Annual bluegrass	+	+	Freitag 1951
Portulaca oleracea	Common purselane	-	- +	Freitag 1951* Wistrom and Purcell 2005*
Ranunculus spp.	Buttercup	-	None	
Rumex crispus	Curly dock	-	+	Freitag 1951
Salsola tragus	Russian thistle	-	None	
Senecio vulgaris	Common groundsel	+	None	
Sisymbrium irio	London Rocket	+	None	
Sonchus oleraceus	Annual sowthistle	-	+	Wistrom and Purcell 2005*
Sonchus spp.	Sowthistle	+	-	Costa et al. 2004
Sorghum halepense	Johnson grass	-	+ -	Freitag 1951* Raju et al. 1980, 1983
Stellaria media	Chickweed	+	None	
Typha spp	Cat tail	-	None	
Urtica urens	Burning nettle	+	None	
Veronica persica	Speedwell	+	None	
Xanthium strumarium	Cocklebur	-	+	Wistrom and Purcell 2005*

Results are compared against previous field surveys near vineyards and riparian areas, except for references marked * which refer to greenhouse studies.

Table 2. Concentrations of *X. fastidiosa* found in almond trees with classical symptoms of almond leaf scorch and in surrounding vegetation.

Site (County)	Strain in almonds	Strain in ground vegetation	*X. fastidiosa* titer[2]
Butte	Almond	Almond	1.84×10^6 ab
Butte	Almond	Almond	2.30×10^6 b
Glenn	Almond	Almond	1.19×10^7 a
Glenn	--	Almond	N/A
Stanislaus	Grape	Grape	2.15×10^7 c
Stanislaus	Almond	Almond	Costa et al. 2004.27 x 10^6 ab
Kern	Almond	Almond	1.08×10^7 a

[1] Results based on PCR and restriction enzyme digestion with Rsa1.
[2] Average CFU/g cultured from ALS symptomatic petioles, July 27 and October 14, 2004.
[3] Different letters after each mean indicate a significance difference ($P < .05$), Tukey's pairwise comparison.

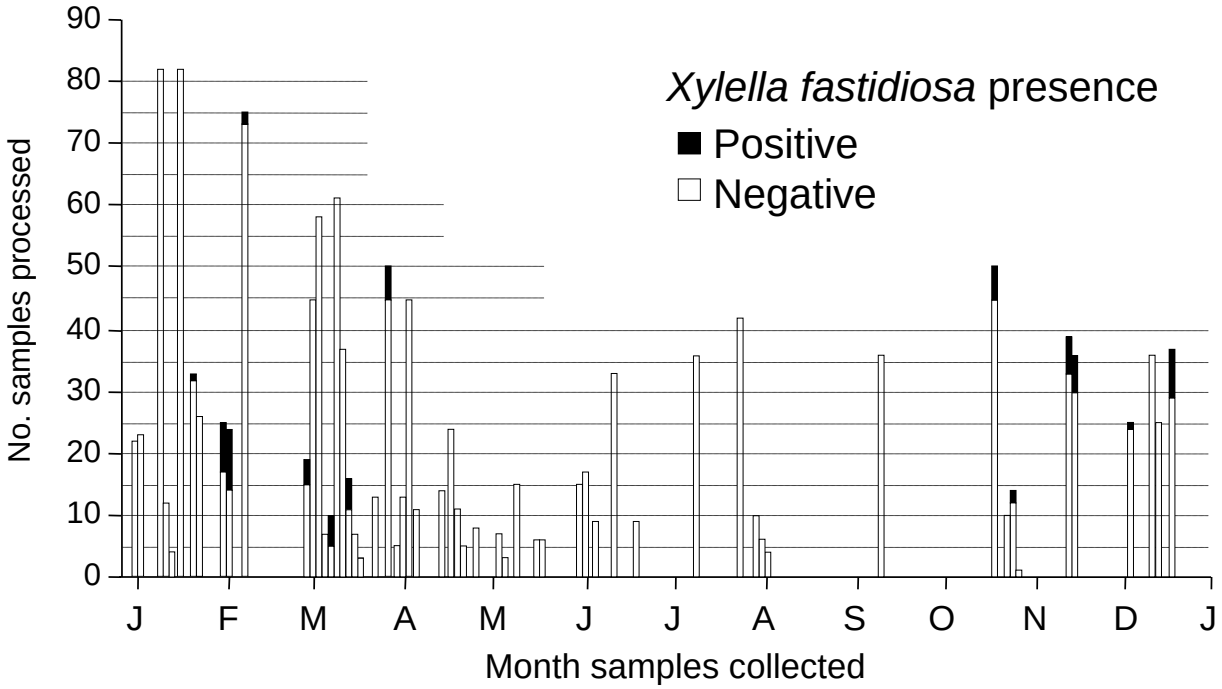

Figure 1: Survey of vegetation in almond orchards for *X. fastidiosa*. Data show results from six almond orchards in Butte, Glenn, Stanislaus, and Kern Counties from June 2003 to April 2005.

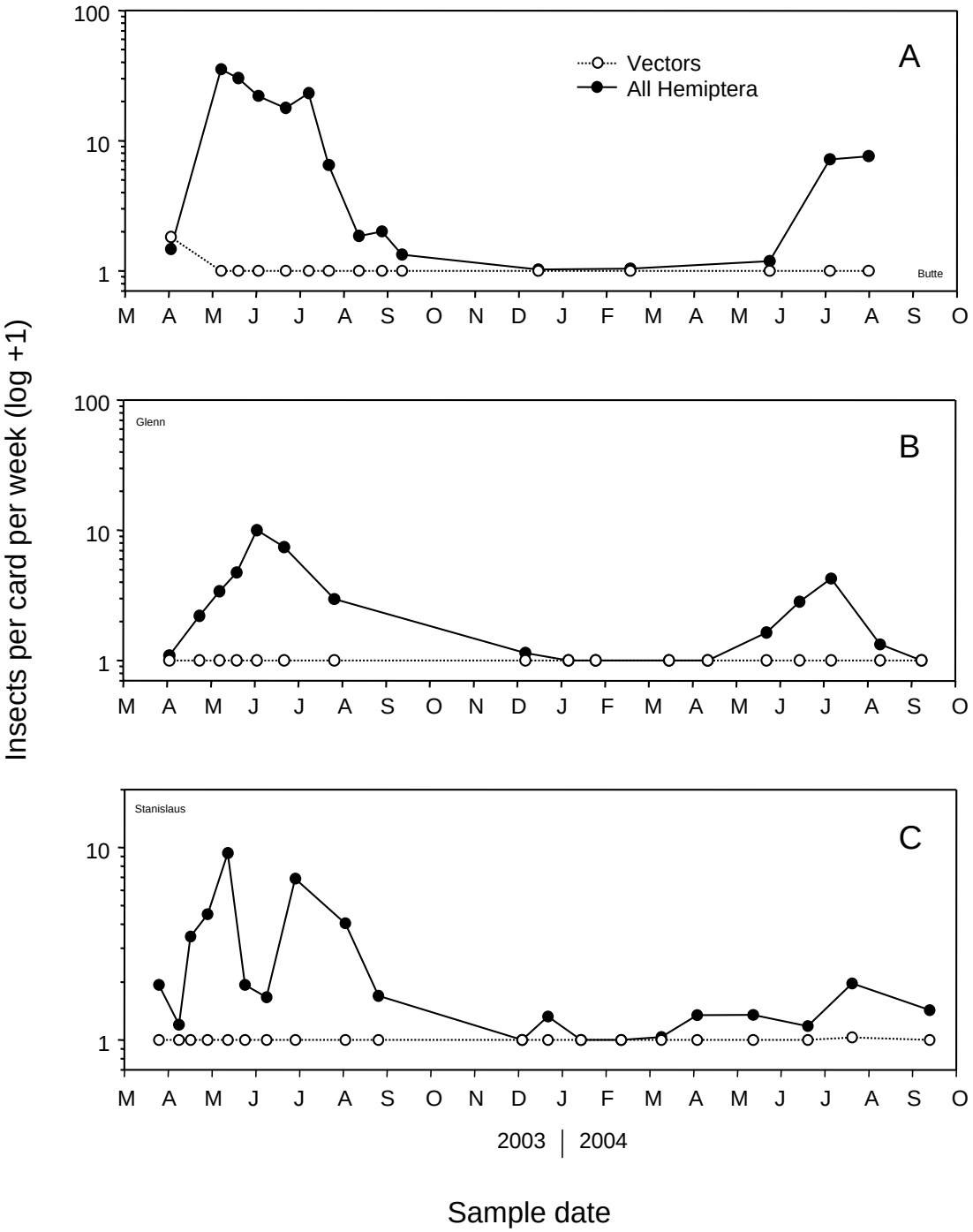

Figure 2. The number of “potential vectors” (hemiptera) and “known vectors” (e.g., sharpshooters and spittlebugs) collected on yellow sticky cards. Data are from three of eight surveyed orchards, representing (A) Butte, (B) Glenn and (C) Stanislaus counties.

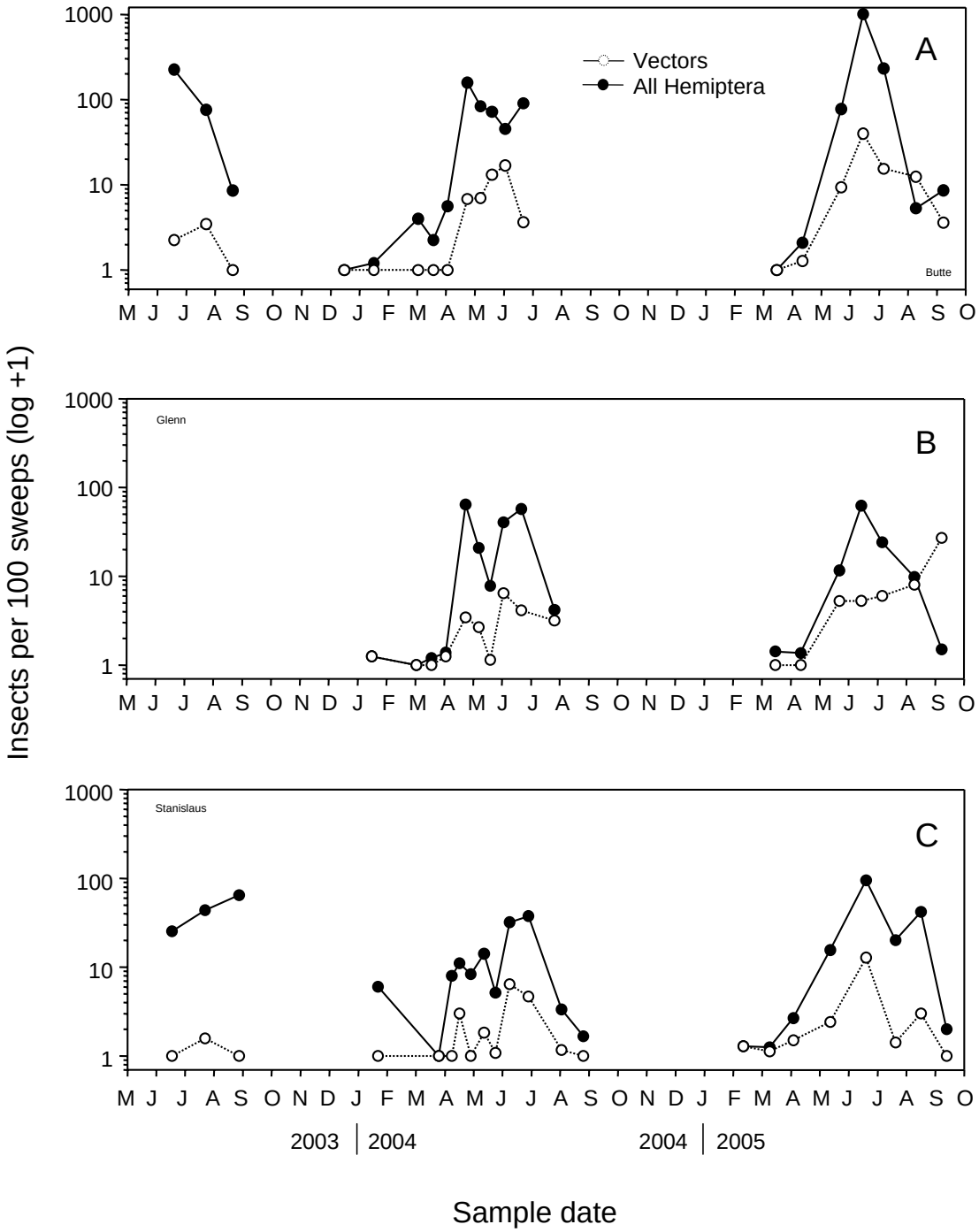

Figure 3. The number of "potential vectors" (hemiptera) and "known vectors" (e.g., sharpshooters and spittlebugs) collected from sweep samples. Data are from three of eight surveyed orchards, representing (A) Butte, (B) Glenn and (C) Stanislaus counties.