# IMPORTANCE OF GROUND VEGETATION IN THE DISPERSAL AND OVERWINTERING OF *XYLELLA FASTIDIOSA*

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## ABSTRACT
Our goal is to determine the ability of alternate host plants, specifically "ground vegetation," in or near almond orchards or vineyards to serve as reservoirs for *Xylella fastidiosa (Xf)*. 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 *Xf* presence using restriction enzyme digestion of RST31-RST33 polymerase chain reaction (PCR) products and bacterial culture on selective media. Overall incidence of *Xf* was low in the ground vegetation species, only 63 of 1369 plant samples tested positive. Of the 37 species of common ground vegetation sampled, 11 tested positive for *Xf*, 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 *Xf* (6 of 7 surveyed sites). ALS-infected almond samples had a *Xf* concentration within reported ranges, however, we were unable to accurately measure *Xf* titer in sampled ground vegetation for comparison.

## INTRODUCTION
The epidemiological factors of Pierce's disease (PD) and almond leaf scorch (ALS), which lead to economic damage, requires more than the presence of susceptible crop cultivars, insect vectors, and plant species suitable for vector feeding and/or breeding. After the pathogen, *Xylella fastidiosa* (*Xf*) is inoculated into a host plant, bacterial multiplication, systemic movement, and expression of disease symptoms depends on many factors including temperature, date of infection, and the *Xf* strain and concentration. We hypothesize that a reservoir population of *Xf* can reside in and around grape or almond orchards without the outward expression of plant disease. *Xf* reservoirs in adjacent vegetation may increase the window of vulnerability for nearby susceptible crops to become infected by providing enough inoculum for vectors at critical periods. For this reason, removal of blue green sharpshooter breeding hosts was an effective method for controlling the spread of PD in coastal wine grape regions. 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. By identifying the seasonal presence and incidence of *Xf* in common ground vegetation in or near almond orchards, weed control efforts can appended to also reduce reservoir *Xf* host species and reduce the level of bacterial inoculum.

We report here are sample collections of annual plant species in almond orchards, where ALS incidence had been recorded for more than 2 years (all sites reporting PD and/or GWSS were heavily treated with insecticides in 2005).

## OBJECTIVE
1. Determine the presence of *Xf* in alternate host plants that are commonly visited by glassy-winged and native sharpshooters in selected ecosystems in the San Joaquin Valley; with samples representing different seasons and annual or perennial hosts.

## RESULTS
### Ground vegetation survey
Surveyed almond orchards were located in California's north Central Valley (Butte Co., Glenn Co.), the middle of the Central Valley (Stanislaus Co.) and the south Central Valley (Kern C.). Every 2 to 6 weeks, depending on the seasonal availability of ground vegetation, a visual survey and collection of the four most abundant weed species was conducted. A total of 58 collections were made. There were 37 species of ground vegetation commonly found (Table 1), with most material collected in winter and spring when ground vegetation was common.

**Bacterial detection and strain identification**
Each sample (orchard site, sample date, plant species, n = 1369) was processed separately for the presence of *Xf*, using immunocapture DNA separation and PCR amplification procedures developed by B.C. Kirkpatrick (UC Davis, pers. comm.). After gel electrophoresis, a preliminary strain difference analysis was carried out according to Minsavage et al. (1994).

Sixty three of 1369 samples from the six orchards were positive for *Xf* (4.6%). *Xf* was recovered from 11 of the 37 ground vegetation plant 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 *Xf* positive samples found between April and mid-October during the two years of the study (Figure 1). Results from both PCR and culture on selective media showed that almond trees in 6 of 7 experimental orchards were infected with the almond strain of *Xf*. At one site (Stanislaus Co.), a grape strain of *Xf* was isolated from all weeds and almond trees sampled. At each site, tissue samples from both almond trees and surrounding weeds was either the grape or almond strain of *Xf*, but never both.

**Bacterial titer and incidence**
Attempts were made to culture *Xf* from symptomatic almond trees, as well as fresh samples of alternate host plants, using procedures described by Hill and Purcell (1997), in order to determine both the strain and concentration of bacteria in almond and ground vegetation samples.

Petioles from ALS infected almond trees containing the grape strain of *Xf* had an average concentration of $2.15 \times 10^7$ CFU/g, which is significantly greater than the concentrations at other sites sampled ($1.84 \times 10^6$ - $1.19 \times 10^7$ CFU/g) ($P = 0.014$). Previous studies also showed average *Xf* titer in ALS-symptomatic almond leaves (Almeida and Purcell 2003) is lower than the average *Xf* titer in PD-symptomatic grapes (Hill and Purcell 1997). All ground vegetation samples were contaminated with other bacteria species and *Xf* presence could be determined. Previous researchers have also encountered difficulty in culturing *Xf* from field samples (Wistrom and Purcell 2005).

**Table 1.** Presence of *Xylella fastidiosa* in ground vegetation in ALS-infected almond orchards (using immunocapture DNA extraction and PCR) in this study are compared against previous field surveys near PD-infected vineyards, except for references marked * which refer to greenhouse studies [1].

| Scientific Name (Common Name) | This study | Other studies | Reference |
|---|---|---|---|
| *Capsella bursa-pastoris* (Shepherd's purse), *Senecio vulgaris* (common groundsel), *Sisymbrium irio (*London rocket), *Stellaria media* (Chickweed), *Urtica urens* (burning nettle), *Veronica persica* (Speedwell), | + | None | |
| *Chamaesyce maculate*, (spotted spurge), *Chenopodium album*, (lambsquarter), (*Conyza bonariensis*, (fleabane), *Coronopus didymus*, (lesser swine cress), *Festuca spp.*, (fescue grass), *Ranunculus spp.*, (buttercup), *Salsola tragus*, (Russian thistle), *Typha spp.* (cat tail) | - | None | |
| *Erodium spp.* (filaree) | + | + | 2* , 4* |
| *Medicago polymorpha* (burclover), *Poa annua* (annual bluegrass) | + | + | 2 |
| *Erodium spp.* (filaree), *Sonchus spp.* (sowthistle), *Malva parvifolia* (cheeseweed), | + | - | 1 |
| *Avena fatua* (wild oat), *Cyperus esculentus* (yellow nutsedge), *Escallonia montevidensis* (escallonia), *Hordeum murinium* (hare barley), *Rumex crispus* (curly dock) | - | + | 2* |
| *Brassicaceae spp.* (mustards), *Helianthus spp.* (sunflower) | - | + | 1 |
| *Claytonia perfoliata* (miner's lettuce) | - | + | 3 |
| *Amaranthus spp.* (pigweed), *Conyza canadiensus* (horseweed), *Echinochloa crus-galli* (barnyard grass), *Lactuca serriola* (prickly lettuce), *Portulaca oleracea* (common purselane), *Sonchus oleraceus* (annual sowthistle), *Xanthium strumarium* (cocklebur) | - | + | 4* |
| *Amaranthus spp.* (pigweed), *Amsinckia spp.* (fiddleneck), *Anagallis arvensis* (scarlet pimpernel) | - | - | 1 |
| *Lactuca serriola* (prickly lettuce), *Sorghum halepense* (Johnson grass) | - | - | 3 |
| *Portulaca oleracea* Common purselane | - | - | 2* |

[a] References cited are 1 = Costa et al. 2004, 2 = Freitag 1951, 3 = Raju et al. 1983, 4 = Wistrom and Purcell 2005

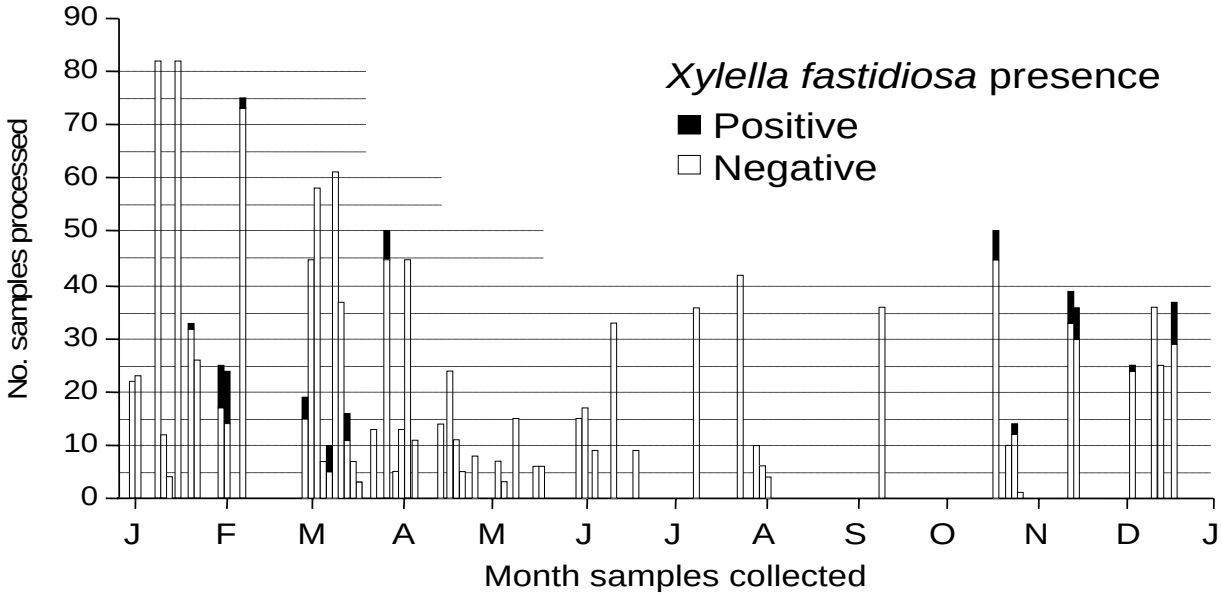


**Figure 1.** Survey of vegetation in almond orchards for *Xf*. Data show combined results from six almond orchards in Butte, Glenn, Stanislaus, and Kern Counties from June 2003 to April 2005.

**CONCLUSIONS**

All previous field surveys for *Xf* 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 *Xf*. This will be of prime importance as GWSS moves northward into areas dominated by nut and vineyard crops. We showed the presence of *Xf* were present in 29.7% of the ground vegetation species sampled. Numerous studies have documented the survival of *Xf* in different plant species; however, fewer have included field surveys (but see Raju et al. 1983, Hopkins and Alderz 1988, Costa et al. 2004), or the season-long incidence of *Xf* in non-symptomatic ground vegetation.

Of the *Xf* 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 *Xf* ($y = 0.0553x - 0.2074$, $r^2 = 0.8935$). Some plant species in the sampled orchards were common hosts of *Xf* in other surveys, but were negative in our 2 year survey (Table 1).

We found the almond strain of *Xf* was most common in the surveyed ALS-infected orchards. Recent studies on the biology of different strains of *Xf* have shown varying abilities to infect different hosts (e.g., Almeida and Purcell 2003). A recent study near Fresno, California, showed that characteristics of different varietals of almonds as well as strain type result in differing severity of ALS (Groves et al. 2005). A parallel study found both the almond and grape genotypes of *Xf* 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 significantly higher *Xf* titers in almond petioles containing the grape strain, as compared to petioles with almond strain *Xf* ($P < 0.014$), as has been reported previously (Hill and Purcell 1997, Almeida and Purcell 2003).

Perhaps most important for the relationship between ALS and PD epidemiology and resident ground vegetation is that we detected *Xf* in weeds only between October and April. Other field surveys, conducted primarily during the growing season, detected *Xf* during the summer (Costa et al. 2004, Freitag 1951, Wistrom and Purcell 2005). Seasonality and temperature is important for ALS or PD epidemiology as *Xf* survives best in the plants at a moderate temperature and plants inoculated on leaf tissue late in the growing season may not develop chronic disease symptoms. Ground vegetation in the surveyed orchards best harbored *Xf* 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. A possible reason for the difference between these studies is that, during the late spring and summer months, most ground vegetation in the almond orchard was small and in poor condition due, in part, to almond management practices of preparing the orchard floor for harvest operations. Therefore, cultural practices may also impact *Xf* levels in alternative host plants.

These results suggest further investigation of the seasonal presence and concentration of *Xf* 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, most previous work has not revealed any clear spatial patterns with ALS. As ground vegetation can harbor *Xf* on the almond floor, our results suggest that a year-round vegetation management may assist in PD or ALS

management. Also, the feeding behavior and plant preference of insects could be a more important factor in controlling the spread of PD and ALS.

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