ASSESSMENT OF THE IMPORTANCE OF ALFALFA TO THE EPIDEMIOLOGY OF XYLELLAE DISEASES IN THE SAN JOAQUIN VALLEY OF CALIFORNIA

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ABSTRACT
The role of alfalfa in the epidemiology of xylellae diseases in the San Joaquin Valley of California was assessed. Alfalfa was investigated as it is a known host of *Xylella fastidiosa (Xf)* and often harbors large populations of a native vector, *Draeculacephala minerva.* Laboratory inoculation of 14 cultivars of alfalfa indicated that all cultivars tested were suitable hosts. The persistence of infections in alfalfa was followed in four cultivars over one year. For plants held outdoors, detection of *Xf* via PCR declined during the winter and increased again during the summer, suggesting that cool winter temperatures decreased titers of *Xf*. Sampling of alfalfa fields seasonally found that incidence of *Xf* in alfalfa was low with only six positive samples detected out of >4,000 screened. All positive samples were collected in summer agreeing with seasonal trends in *Xf* detection observed in controlled studies. Abundance of *D. minvera* in alfalfa was high, although the highest numbers were caught on traps located on weedy field margins. Preference of *D. minerva* for weeds in alfalfa fields would limit the spread of *Xf* in alfalfa. The results indicate that alfalfa can serve as a source of vectors, but its role as an inoculum source is unclear. Future work should determine the incidence of *Xf* in weeds commonly found in alfalfa fields that are preferred feeding hosts of *D. minerva.*

INTRODUCTION
Pierce's disease of grape and almond leaf scorch disease has been chronic problems in California's San Joaquin Valley. In the San Joaquin Valley of California, the green sharpshooter (*Draeculacephala minerva)* is thought to be the most important vector. Green sharpshooters are often abundant in alfalfa fields and alfalfa is a known host of *Xylella fastidiosa (Xf).* Alfalfa is thought to play an important role in the epidemiology of xylellae diseases because alfalfa is often planted in proximity to almond and grape and clusters of diseased almond trees and grape vines are often observed on orchard or vineyard edges which border alfalfa. Due to the large acreage planted with alfalfa in the San Joaquin Valley, its potential to serve as a host of *Xf,* and its propensity to harbor vectors, we initiated studies to assess the role of alfalfa in the epidemiology of xylellae diseases.

OBJECTIVES
1. Estimate *Xf* incidence in forage alfalfa planted adjacent to grape and/or almond.
2. Characterize the seasonal abundance and dispersal of green sharpshooters present within and emigrating from alfalfa.
3. Determine the relative susceptibility of selected alfalfa cultivars to infection by *Xf.*

RESULTS
Objective 1. Estimate Xf incidence in forage alfalfa planted adjacent to grape and/or almond. We sampled alfalfa fields in Fresno, Tulare, and Kern counties seasonally (winter, spring, summer & fall) to estimate incidence of *Xf* starting in summer of 2005 to present. To date, >4,000 samples have been screened for the presence of *Xf* using conventional PCR (Minsavage et al. 1994). Of those samples, six have been confirmed positive. Two positives came from a collection in Fresno County during the summer of 2005 (**Figure 1**). The other four positives came from another collection in Fresno County during the summer of 2007.

Objective 2. Characterize the seasonal abundance and dispersal of green sharpshooters present within and emigrating from alfalfa. The abundance and spatial distribution of the green sharpshooter was monitored in alfalfa fields in Fresno, Kern, and Tulare counties throughout 2006, 2007, and 2008. Four transects of yellow sticky traps were placed in each field. Traps were counted and replaced biweekly. Preliminary analysis of the distribution of green sharpshooter within alfalfa fields indicates some important trends. First, green sharpshooters were more abundant on field edges than in the middle of fields (**Figure 2A**). Similarly, the number of insects caught per trap was often associated with the percentage of ground cover that was weeds (**Figure 2B**). Together, this indicates that *D. minerva* adults prefer weeds that are found along field margins.

Objective 3. Determine the relative susceptibility of selected alfalfa cultivars to infection by Xf. Fourteen alfalfa cultivars were screened to determine their relative susceptibility to infection by four different *Xf* strains (Temecula, Dixon, M12, and M23). Plants were screened for infection using conventional PCR methods 12 weeks after inoculation. *Xf* was detected in at least three out of 24 plants for each cultivar and the percentage of plants infected averaged across the four strains varied from 13 to 48%.

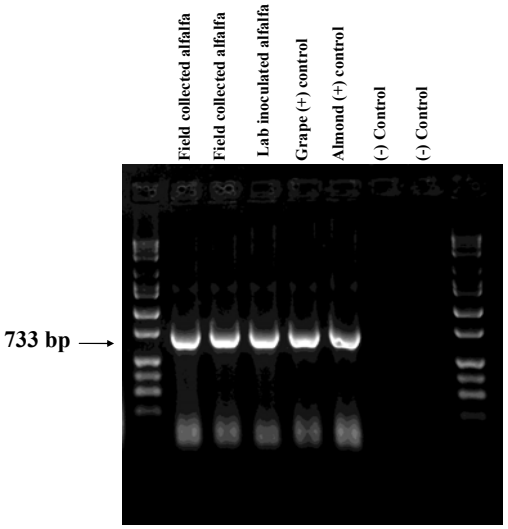

Figure 1. Examples of alfalfa positives detected using RST 31/33.

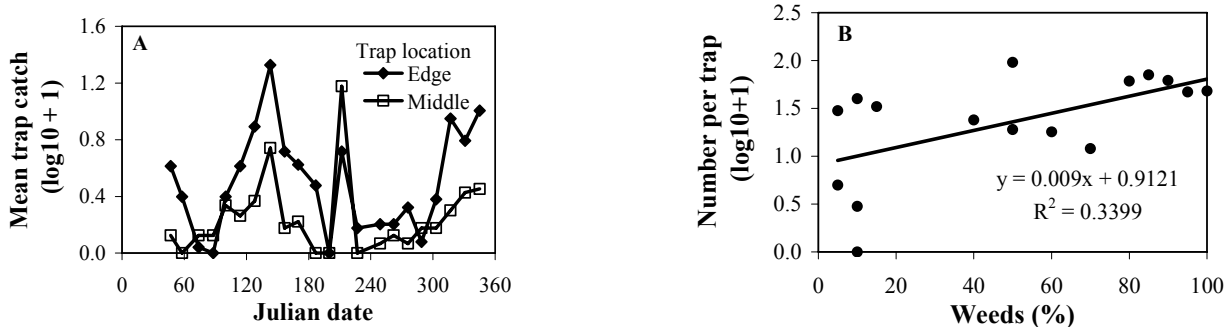

Figure 2. Examples from preliminary analysis of trap catch data. A) Mean number of *D. minerva* caught on sticky traps located on the edge of an alfalfa field verses the middle of an alfalfa field in Fresno County during 2007. B) Association of the number of *D. minerva* caught per trap between January 8, 2007 and August 3, 2007 and the percentage of ground cover surrounding a trap that was weeds for an alfalfa field in Kern County.

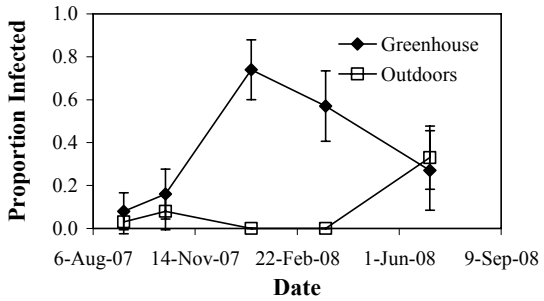

Figure 3. Incidence of *Xf* in needle-inoculated plants held in the greenhouse versus outdoors. All plants were inoculated on the same date in July of 2007.

For 5 cultivars (CUF 101, Moapa 69, WL 530 HQ, WL 625 HQ, and WL 342 HQ) a more detailed experiment was conducted to determine the seasonal fate of *Xf* in alfalfa. Approximately 20 plants of each cultivar were needle inoculated in July of 2007. Half of the plants were held outdoors in a screen cage and the other half were held indoors in a greenhouse. Plants were screened for the presence of *Xf* regularly using standard PCR methods. Screening of samples in October of 2007 indicated no differences between plants held outdoors versus those held in the greenhouse (**Figure 3**). However, by January of 2008 all samples from plants held outdoors were negative for *Xf* whereas most samples from plants held in the greenhouse were positive (**Figure 3**). This suggests that cool winter temperatures reduced the titer of *Xf* in plants held outdoors. By July of 2008, the incidence of *Xf* was the same for both sets of plants suggesting that cool winter temperatures did not

eliminate infections from plants held outdoors, but simply reduced *Xf* titers to levels that were not detectable via PCR. These results indicate important seasonal fluctuations in *Xf* titer.

CONCLUSIONS

All alfalfa cultivars tested were suitable hosts for *Xf* and green sharpshooters were abundant in alfalfa fields (**Figure 2**). Incidence of *Xf* in field collections averaged over all sites and dates was low (six out of >4,000 samples tested) and all *Xf* positive samples were collected during the summer. Monitoring of needle inoculated plants held throughout the year suggest that *Xf* titers decline during the winter (**Figure 3**), supporting the observation that *Xf* positive alfalfa samples were collected only in the summer. Trapping of *D. minerva* in alfalfa fields indicates that they prefer weedy field margins and likely feed preferentially on weeds versus alfalfa. If true, this would limit the spread of *Xf* in alfalfa. The results suggest that alfalfa can serve as a source of vectors, but that the role of alfalfa as an inoculum source is unclear. Future work should focus on examining the incidence of *Xf* in weeds commonly found in alfalfa fields that are preferred feeding hosts of *D. minerva.*

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Minsavage, G. V., C. M. Thompson, D. L Hopkins, R.M.V.B.C. Leite, and R.E. Stall. 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84: 456-461.

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