ASSESSING THE POTENTIAL OF FORAGE ALFALFA CROPS TO SERVE AS PIERCE'S DISEASE PRIMARY INOCULUM SOURCES IN THE SAN JOAQUIN VALLEY

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ABSTRACT

The potential for forage alfalfa to serve as a primary inoculum source of Pierce's disease (*Xylella fastidiosa; Xf*) in the San Joaquin Valley of California was evaluated. Laboratory inoculation of fourteen cultivars of alfalfa indicated that all alfalfa cultivars tested were equally suitable hosts for *Xf.* Incidence of *Xf* in forage alfalfa averaged across all field sites located in the San Joaquin Valley of California was low (0.1%), although incidence in one field on one date was 9%. Green sharpshooter (*Draeculacephala minerva*), a known vector of *Xf*, was prevalent at field sites and preferred field edges. The three cornered alfalfa hopper (*Spissistilus festinus*), whose vector competency is unknown, was more abundant in field samples than green sharpshooter. Greenhouse transmission tests with the three cornered alfalfa hopper have not documented competent transmission. The results indicate that alfalfa has the potential to be an important inoculum source due to its suitability as a host for *Xf* and the presence of vectors, but that measured incidence of *Xf* in alfalfa is typically low.

INTRODUCTION

Pierce's disease (PD) of grape and almond leaf scorch disease threaten grape and almond production in California's San Joaquin Valley. Little is known about where potential insect vectors acquire the pathogen, when they move into orchards/vineyards, and when they spread the pathogen. Alfalfa is widely planted in the Southern San Joaquin Valley and alfalfa fields often border vineyards and almond orchards. Alfalfa is a host of *Xylella fastidiosa (Xf)* and often harbors high numbers of known vectors (Freitag and Frazier 1954). Due to the large acreage planted to this crop in the San Joaquin Valley, it's potential to serve as a host of *Xf,* and it's propensity to harbor vectors, we initiated studies to quantify the potential of alfalfa forage crops to serve as a primary source of *Xf* inoculum.

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.*
4. Determine the vector competence of a potentially new insect vector, the three-cornered alfalfa hopper, *Spissistilus festinus*, (Hemiptera: Membracidae).

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 County seasonally (Winter, Spring, Summer, Fall) to estimate the incidence of *Xf* starting in the summer of 2005 to the present. To date, 5,533 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. 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 and 2007. Four transects of yellow sticky traps were placed in each

field. Traps were counted and replaced biweekly. Peaks in trap catches occurred in May/June and in July/August (Figure 1). Trap catches were generally higher on field edges than in the middle (Figure 1).

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). Six plants of each cultivar were needle inoculated with each strain of *Xf*. Plants were screened for infection using conventional PCR 12 weeks after inoculation. The pathogen was detected in at least three out of 24 plants for each cultivar and the percentage of plants infected averaged across the four *Xf* strains varied from 13 to 48% (Figure 2). After screening, only infected plants and control plants were kept. Plants were screened again in early fall and infections were confirmed in 35% of plants previously determined to be infected.

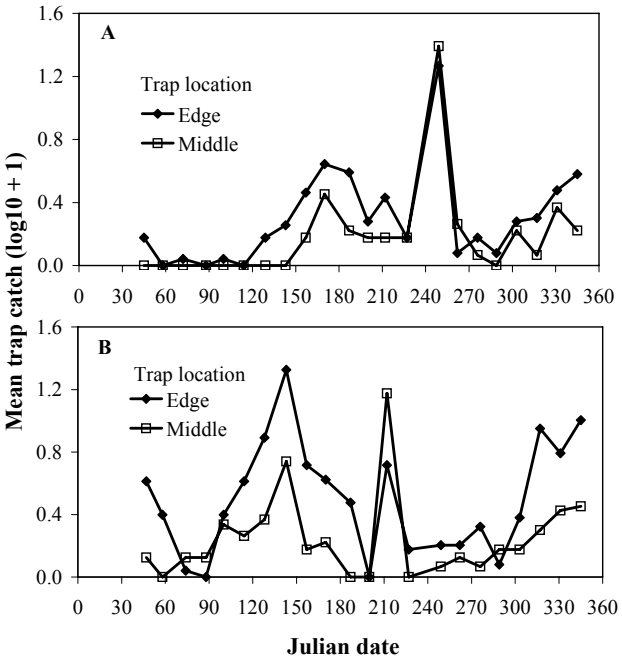

Fig. 1. Trap catches of green sharpshooter in alfalfa at sites located in (A) Tulare and (B) Fresno Counties. Traps were located in the middle of fields or on edges.

Objective 4. Determine the vector competence of a potentially new insect vector, the three-cornered alfalfa hopper, *Spissistilus festinus*.

Transmission assays were conducted in August of 2006. Three needle inoculated alfalfa plants were used as acquisition hosts. Approximately, 150 *S. festinus* adults were collected from the field and placed in a cage with *Xf* source plants for a four day acquisition access period (AAP). After the four day AAP, insects were caged in groups of five on uninfected alfalfa for a 4 day inoculation access period (IAP). After the four day IAP, all insects were frozen and plants held. This experimental protocol was repeated three times and a total of 430 *S. festinus* were tested on 86 test plants (86 plants x five insects per plant = 430). Plants were screened using conventional PCR 3 months and 5 months after the IAP for each experimental replicate. PCR screening failed to detect the presence of *Xf* in test plants and attempted cultures of *Xf* from a subset of plants also detected no infections. A subset of *S. festinus* heads was screened via PCR for the presence of *Xf* with no positives. Results of this experiment do not suggest that *S. festinus* is a competent vector of *Xf*. This experiment is currently being repeated.

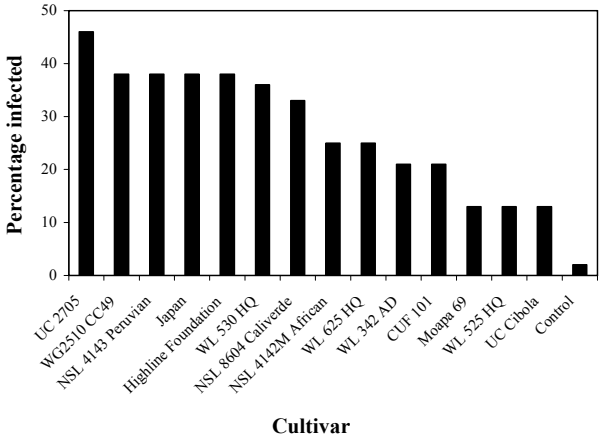

Fig. 2. Percentage of each alfalfa cultivar succesfully needle incoulated with *Xf* across all *Xf* strains tested.

CONCLUSIONS

All alfalfa cultivars tested were suitable hosts for *Xf* (Figure 2) and green sharpshooters were abundant in alfalfa fields (Figure 1). Incidence of *Xf* in field collections averaged over all sites and dates was low (6 out of 5,533 of tillers tested = 0.1%). However, four of six positive samples came from a single collection of 45 tillers collected from a field in Fresno County during the summer of 2007, giving an incidence of 9% in that field on that date. This suggests that incidence of *Xf* in alfalfa may typically be low, but that there may be localized 'hot spots' in some locations during some years. This observation combined with high vector activity in alfalfa suggests that continued investigation into the role of alfalfa in the epidemiology of xylellae diseases is warranted.

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