ROLE OF ALFALFA IN THE EPIDEMIOLOGY OF *XYLELLA FASTIDIOSA* IN CALIFORNIA

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ABSTRACT
Alfalfa (*Medicago sativa*) occurs widely throughout the Central Valley, often adjacent to grape and other plants susceptible to *Xylella fastidiosa* (*Xf*). Although a previous epidemic of Pierce's disease (PD) in the Central Valley was associated with migration of infective insects from alfalfa fields to vineyards, little is known about alfalfa-*Xf* interactions and their importance to disease epidemiology. In this project we are studying the suitability of alfalfa as a perennial host of PD and almond leaf scorch (ALS) strains of *Xf* and its role as a source of the pathogen for vector transmission. In a first study, we showed that several isolates of each strain can colonize alfalfa after mechanical inoculation, although the rate of infection and bacterial population over successive cuttings varied depending on the isolate. We are now testing transmission of an isolate of each strain to/from alfalfa to grape and almond by the green sharpshooter (*Draeculacephala minerva*), as well as studying the feeding sites of three sharpshooter vectors in relation to the distribution of *Xf* in the alfalfa plant. These studies should provide basic information on vector-alfalfa-*Xf* interactions, which may be considered in management strategies for pathosystems that include alfalfa as a host.

INTRODUCTION
In recent years, many aspects of *Xf* diseases have been studied, providing information that can be used to develop management practices. However, some aspects of the epidemiology of diseases like PD and ALS are poorly understood, such as the role of alfalfa (*Medicago sativa*) in the maintenance and spread of *Xf* in California. This bacterium colonizes alfalfa and it was associated with the alfalfa dwarf (AD) disease (Thomson et al. 1978). Alfalfa occurs throughout the Central Valley and it was directly associated with the largest PD epidemic that had occurred in this region (Hewitt et al. 1949) until the introduction of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*. Studies on the epidemics of the 1940s showed that PD was more frequent in areas adjacent to alfalfa fields, and that a disease gradient was observed, in which PD incidence was higher near alfalfa fields (Hewitt and Houston 1941). It was later found that the etiological agent of AD was transmitted by sharpshooter leafhoppers (Hewitt et al. 1946) and that spread to adjacent grapevines was associated to two sharpshooters commonly found on Bermuda grass and alfalfa fields, *Xyphon fulgida* (red-headed sharpshooter, RHSS) and *Draeculacephala minerva* (green sharpshooter, GSS) (Purcell and Frazier 1985).

In relation to PD, interest in AD was primarily focused on its importance as a reservoir of *Xf* to grape rather than studies aimed to understand the alfalfa-*Xf* association. Weimer described AD in a series of publications (Weimer 1932, 1936, 1937a), but at that time the disease was presumed to be caused by a virus (Weimer 1937b). Focus on AD was briefly renewed when Davis et al. were able to culture *Xf* from infected grape and almond plants (1978, 1980). Thomson et al. (1978) were also able to culture *Xf* from alfalfa, although Koch's postulates were not fulfilled for the disease. Since then, no further information was reported on AD. We do no know which *Xf* strains cause disease in alfalfa, or which strains can multiply in this plant. The only work done on this aspect of the disease showed that *Xf* isolated from alfalfa caused disease in almond (Thomson et al. 1978). Recently, it was shown that grape and almond *Xf* are genetically and biologically different (Almeida and Purcell 2003). Grape strains cause disease in grape and almonds, whereas almond strains do not cause disease in grape. This is of paramount importance in explaining the movement of *Xf* from vineyards to almond orchards and vice-versa. However, the role of alfalfa in this pathosystem remains unclear, as we do not know if these strains multiply in alfalfa to numbers high enough for insect acquisition and subsequent transmission to grape or almond. In this project we are evaluating the fate of grape and almond strains of *Xf* in alfalfa, as well as their acquisition and inoculation by vectors on this host plant, in order to determine the importance of alfalfa crop as a source for these strains.

OBJECTIVES
1. Assess multiplication, movement and survival of grape and almond strains of *Xf* in alfalfa.
2. Evaluate pathogenicity of *Xf* strains to alfalfa.
3. Determine vector transmission efficiency of *Xf* to/from alfalfa to grape and almond.

RESULTS
Objective 1. Colonization of alfalfa by grape and almond strains of *Xf*
In this study, we are assessing the multiplication and survival in alfalfa (cv. WL625HQ) of 12 isolates of *Xf* from grape, 10 isolates from almond and two from alfalfa (Table 1). Potted alfalfa seedlings were pin-inoculated in the stem (2" above soil

level) with a 10^8-10^9 CFU/mL suspension of each isolate; we kept the plants in a vector-proof greenhouse and cut at 1.5" above soil after each flowering stage. *Xf* infection and concentration at the base of the stem was determined by primary isolation on solid PWG medium (Hill & Purcell 1995) at 8, 14 and 21 wks after the inoculation (just before the 1st, 2nd and 3rd cuts, respectively). At each cut, plants were evaluated for height, number of internodes and harvested dry mass, in order to determine strain pathogenicity to alfalfa. One last evaluation of *Xf* infection and alfalfa growth will be carried out at 35 wks after inoculation (before the 5th cut).

The results obtained so far show that both grape and almond strains of *Xf* can multiply and survive in alfalfa for at least three cuts. The percentage of infected plants varied widely for grape (0-100%) and almond (11-100%) strains, and the mean bacterial concentrations ranged from 10^6 to 10^8 CFU/g of alfalfa tissue for both strains (Table 1). By the 3rd cut, however, most grape isolates showed mean concentrations around 10^8 CFU/g, whereas concentrations of almond isolates were generally 10 times lower. Reductions in plant height and dry mass in relation to healthy controls (mock-inoculated plants) were apparent at the 3rd cut for at least four grape isolates and one of alfalfa (Figure 1). Statistical analyses for testing the effects of different isolates on alfalfa growth parameters will be carried out after the 5th cut.

Objective 2. Distribution of *Xf* strains in alfalfa in relation to vector feeding sites

We are conducting another study to determine bacterial movement and distribution in relation to vector feeding sites in alfalfa. Initially, a choice experiment including three sharpshooter species [GSS, GWSS and the blue-green sharpshooter (BGSS), *Graphocephala atropunctata*] was carried out to examine vector preference for feeding on different parts of the alfalfa plant. This choice experiment showed that GSS feeds both on the basal and upper portions of the stems, while GWSS prefers to feed on the stem at the medium and upper part of the plant and BGSS feeds exclusively on the upper part of the plant (leaves and stems). Based on the vector seeding sites, we designed a second experiment to measure the movement and distribution of two almond and two grape isolates of *Xf* in alfalfa stems. Potted alfalfa seedlings were mechanically inoculated with cell suspensions of each isolate, as described above. At 6 (1st cut) and 12 (2nd cut) wks after inoculation, the bacterial concentrations at the base of the inoculated stem, in the tap root, and at the base and tip of an adjacent stem will be determined by culturing. As an additional measurement of systemic movement, we will confine healthy sharpshooter vectors at the base and tip of the adjacent stem for an acquisition access period (AAP) and then tested for transmission to indicator plants and assayed for infectivity by PCR. Final results are pending.

Objective 3. Transmission efficiency of *Xf* strains to/from alfalfa

A third study is being carried out to evaluate transmission efficiency by GSS of a grape and an almond isolate of *Xf* to/from alfalfa to grape and almond plants. Healthy adults of GSS were confined on source plants of these isolates for a 48-h AAP and then transferred to test plants of alfalfa, grape and/or almond for an inoculation access period of 48 h. For the grape isolate, alfalfa, almond and grape are being tested as source and test plants, whereas for the almond isolate (not pathogenic to grape), only alfalfa and almonds are being tested. These experiments were already set up, but the results are not available yet.

CONCLUSIONS

Grape and almond isolates multiply and persist in alfalfa to population levels $\geq 10^7$ CFU/g of tissue, which exceed the minimum threshold (10^4 CFU/g) required for *Xf* acquisition by sharpshooter vectors (Hill & Purcell 1997). *Xf* infection reduces height and dry mass of alfalfa. Pending experiments will determine the importance of alfalfa as a source of *Xf* for vector acquisition.

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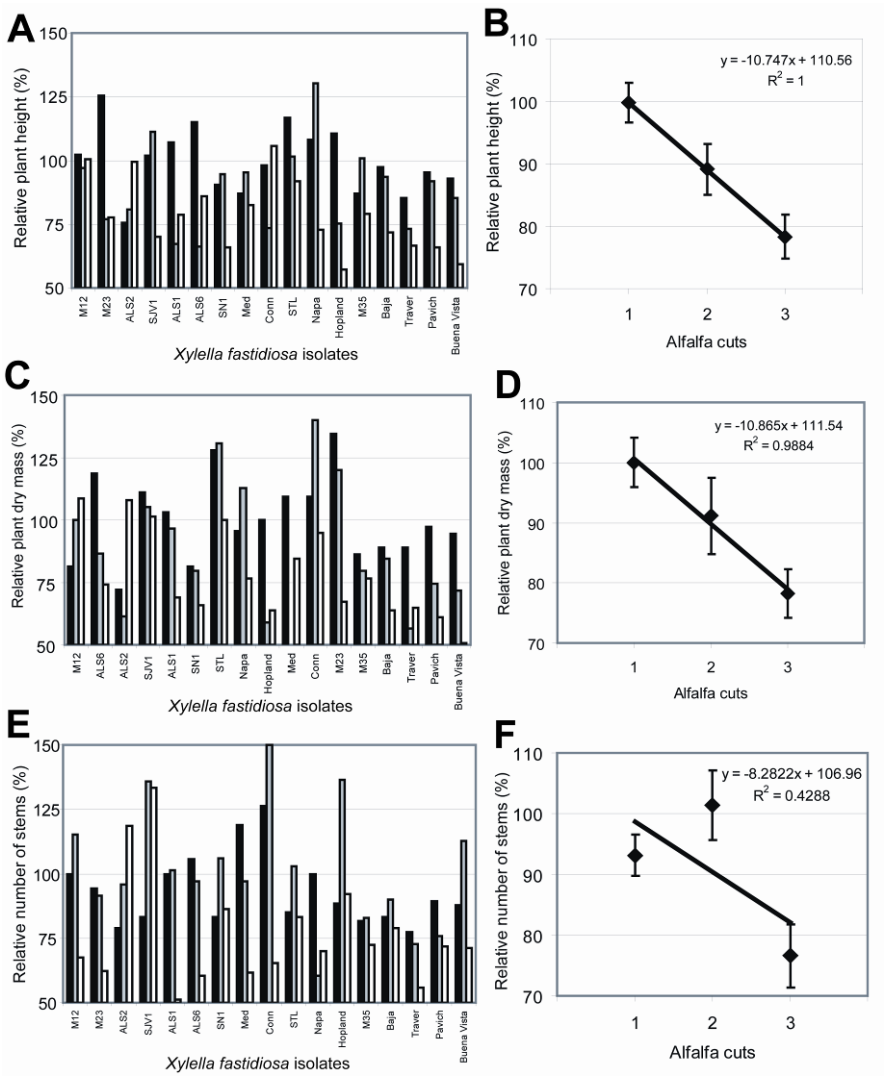

Figure 1. Growth parameters of infected alfalfa plants at successive cuttings following mechanical inoculation of *Xf* isolates. A) Plant height in relation to healthy control; B) Regression of number of cuts versus relative plant height; C) Dry mass in relation to healthy control; D) Regression of number of cuts versus relative dry mass; E) Number of stems in relation to healthy control; F) Regression of number of cuts versus relative number of stems. Black, gray and white columns represent the 1st, 2nd and 3rd cuts, respectively (graphs A, C and E). In graphs B, D and F, means (±SE) are based on pooled data of all isolates.

Table 1. Rate of infection and bacterial concentration of *Xf* isolates in plants of *Medicago sativa* at successive cuttings after mechanical inoculation.

Inoculation date Isolate (County)	Host of origin	Proportion of infected plants 1st cut (8 WAI[a])	 2nd cut (14 WAÍ)	 3rd cut (21 WAI)	Log CFU/g tissue (±SE) 1st cut (8 WAÍ)	 2nd cut (14 WAÍ)	 3rd cut (21 WAÍ)
03/15/2007							
Hopland (Mendocino)	Grape	6/12[b]	1/7	100.0 (9/9)	6.6±0.5	8.0	8.6±0.2
Napa silverado (Napa)	Grape	5/11	4/9	44.4 (4/9)	6.7±0.6	7.8±0.4	8.2±0.4
STL (Napa)	Grape	7/9	4/8	57.0 (4/7)	7.2±0.4	8.7±0.2	7.6±0.5
SN1 (?)	Alfalfa	7/9	6/8	87.5 (7/8)	8.1±0.5	8.6±0.2	7.9±0.3
M12 (?)	Almond	7/9	5/8	44.4 (4/9)	6.3±0.4	7.6±0.2	7.3±0.5
Mock	-						
03/30/2007							
M23 (?)	Grape	7/7	7/7	6/7	8.0±0.4	8.8±0.2	8.6±0.1
Medeiros (Fresno)	Grape	6/6	6/9	6/6	8.1±0.1	8.6±0.2	8.4±0.2
Conn (Napa)	Grape	9/9	4/8	4/8	7.3±0.4	6.5±0.5	7.4±0.5
ALS1 (San Joaquin)	Almond	8/8	8/8	7/7	7.9±0.2	8.2±0.2	8.7±0.2
ALS6 (San Joaquin)	Almond	7/7	6/8	5/7	7.1±0.3	7.9±0.4	7.4±0.2
Butte (Butte)	Almond	9/9	9/9	Ct[c]	8.3±0.2	7.6±0.3	-
ALS4 (San Joaquin)	Almond	7/7	4/7	1/8	7.7±0.2	6.8±0.2	7.7
ALS9 (San Joaquin)	Almond	6/9	5/8	Ct[c]	8.0±0.2	7.6±0.4	-
Mock	-						
04/10/2007							
Buena Vista (Kern)	Grape	9/9	9/9	9/9	8.4±0.2	8.4±0.1	8.7±0.1
Traver (Tulare)	Grape	8/9	9/9	7/7	8.7±0.0	8.6±0.1	8.8±0.2
Pavich (Kern)	Grape	8/9	9/9	9/9	8.3±0.1	8.6±0.2	8.2±0.1
Baja#5 (Mexico)	Grape	6/9	7/8	7/9	7.3±0.2	8.1±0.3	8.7±0.1
Temecula (Riverside)	Grape	1/9	0/8	2/7	5.2	-	8.4±0.5
UCLA (Los Angeles)	Grape	5/9	0/9	1/9	6.3±0.3	-	5.7
M35 (?)	Alfalfa	9/9	7/9	6/7	8.3±0.1	8.0±0.6	7.6±0.4
SJV1 (San Joaquin)	Almond	4/9	2/7	5/9	6.0±0.2	7.3±0.5	7.2±0.6
ALS2 (San Joaquin)	Almond	6/9	3/9	4/9	7.4±0.2	7.8±0.4	7.1±0.3
Glenn (Glenn)	Almond	6/9	5/8	1/9	7.9±0.2	8.4±0.2	5.3
Dixon (Solano)	Almond	3/9	3/9	1/9	7.5±0.2	8.3±0.5	8.0
Mock	-						

[a]WAI: weeks after inoculation.
[b]Number of infected plants over the total number inoculated.
[c]Data not obtained due to plate contamination during bacterial isolation.