Transmission of *Xylella fastidiosa* by *Carneocephala fulgida,* and its survival in Bermuda grass (*Cynodon dactylon*)

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Abstract The ability of Bermuda grass (*Cynodon dactylon*) to retain the bacteria, *Xylella fastidiosa,* can influence the spread Pierce's disease (P.D.). The fate of strains of the bacterium *Xylella fastidiosa* that causes Pierce's disease of grapevines was investigated in Bermuda grass. The P.D. strain, STL, was first mechanically inoculated into Bermuda grass, and was recovered at 1 week, 3 weeks, and 6 weeks after inoculation in the inoculated node only. Following STL, 4 other strains of *X. fastidiosa* (Mederios, SJV, Comm Creek, Preston) were recovered after mechanical inoculation at 3 weeks after inoculation. Three of these strains were recovered again after 6 weeks (Comm Creek, Mederios, Preston). Again detection only occurred in the inoculated node. Insect transmission efficiency of *X. fastidiosa,* by the RHSS (*Carneocephala fulgida)* was also investigated. Seven groups of RHSS' out of 40 groups successfully transmitted the bacterium from infected grape to healthy grape. The seven groups of RHSS had to ability to retain the bacterium after feeding on Bermuda grass.

Introduction

Xylella fastidiosa is a bacterium that is known to be the cause of Pierce's disease (PD), a lethal disease of grapevines (Davis et al. 1978). The bacterium can infect most commercial European grape species, yet some hybrids with American wild grape species are tolerant or resistant to *X. fastidiosa*. *X. fastidiosa* is not limited to grapevines. It infects, usually without symptoms, a wide variety of plant species (Freitag 1951). Studies have shown that in coastal California, the incidence of Pierce's disease of grapevines is high along riparian areas adjacent to vineyards. In the California central valley, P.D. is most intense near bayfields and pastures. (Goodwin 1992, Hill 1995, Purcell 1975). *X. fastidiosa* resides in the xylem of host plants, thus any piercing, sap-feeding insect is a potential vector (Freitag 1954). One of these vector insects is the redheaded sharpshooter (RHSS) *Carneocephala fulgida*. The RHSS feeds primarily on Bermuda grass (*Cynodon dactylon)* (Hewitt et al. 1942), but does have the ability to feed on grapevines.

Bermuda grass is a common weed throughout California. It is a low-growing perennial herb that has been reported as one of the world's 10 worst weeds, having the capacity to grow virtually anyplace with mild winters (Mitich 1989). It has been shown that many plants common to the Californian riparian area can harbor *X. fastidiosa*, yet show no symptoms of disease (Purcell 1999). *X. fastidiosa*'s ability to reside in numerous plants increases the potential for insect vectors to transmit the bacterium to commercial vineyards. With Bermuda grass in close proximity to the vineyards, questions can be raised about its impact in the transmission, spread and retention of *X. fastidiosa*. Bermuda grass is highly sensitive to frost, and low temperatures (Satorre 1996). In the winter Bermuda grass remains inactive, and sprouts again in the spring when the temperature is warmer. It is during spring months that *X. fastidiosa* has been found to be more persistent after insect inoculation in grapes *Vitis vinifera* (Purcell 1981). However, if either grapevines or Bermuda grass plants are infected with *X. fastidiosa,* there is potential for its spread and retention no matter the season.

In a previous study *X. fastidiosa* had been recovered from Bermuda grass by laboratory vector transmission and by field vector recovery (Freitag 1951). Laboratory vector transmission entailed allowing infective RHSS to feed on Bermuda grass, removing them, and then allowing non-infective RHSS to feed. Field vector recovery entailed evaluating RHSS after caging them on field populations of Bermuda grass. The method of detection for this experiment was

questionable. Detection was observed by allowing potentially infected adults to hatch eggs, and allowing the nymphs to feed on healthy grape or alfalfa. Since grape and alfalfa plants display symptoms of bacterial infection, positive detection was determined through symptoms on these plants. Yet there was no clear method of distinguishing if infected adults were actually infective. Also there was room for error when transferring insects to healthy plants. One single leafhopper that was infective could have contaminated a healthy plant that otherwise would have proven to be negative for the bacterium.

In another study it was found that Bermuda grass did not retain any *X. fastidiosa*, when tested by culture and ELISA at the inoculation point 6 to 12 weeks after inoculation (Hill and Purcell 1995). These experiments used 2 strains of *X. fastidiosa*, and possibly were tested too late after inoculation. Further experiments showed that different PD strains of *X. fastidiosa* infected Bermuda grass (Purcell Unpublished data). According to a later study conducted by Purcell and Saunders, populations of *X. fastidiosa* are highest in most plant species within 3 to 6 weeks of inoculation (Purcell 1999).

Our objectives were to:

1. Determine populations of *X. fastidiosa* over time in Bermuda grass after mechanical and vector inoculation.
2. Determine if nine different strains of *X. fastidiosa* differed in their ability to reside in Bermuda grass.
3. Determine if the RHSS could recover *X. fastidiosa* from infected grape and Bermuda grass.

Methods

Systemic growth in Bermuda grass To determine if growth of *X. fastidiosa* was systemic in Bermuda grass, stems were evaluated at two places, once in the inoculated node and then in the next distal node. Testing at two different places on a single node enabled for detection of growth and spread of *X. fastidiosa*. Stems were evaluated at 1, 3, 6 and 9 weeks to determine the survial of *X. fastidiosa* in Bermuda grass. Since *X. fastidiosa* had not been detected past 9 weeks (Hill and Purcell 1995, Purcell unpublished data), these intervals provide new information to the growth and spread of the bacterium in Bermuda grass. The population of Bermuda grass used in

this experiment was cloned from a single plant, which tested negative for the bacterium. All insect-rearing plants were maintained in greenhouses, constantly ventilated with charcoal-filtered air. 25 two-inch plastic pots each with four Bermuda grass cuttings were planted 3 weeks prior to inoculation. Each stem was inoculated on March 31, 2000 with a known strain of *X. fastidiosa* (STL) just above the second node, but below the third node. Inoculation was conducted by dropping 3 microliters of a buffer solution containing the bacterium onto each stem, and puncturing the stem to allow absorption of the bacteria. Each stem was punctured 5 times with an #2 entomology needle. The solution contained cell suspensions of *X. fastidiosa* diluted in SCP buffer to approximately 10^8 CFU/mL (colony forming units per milliliter). The factor of dilution was determined from a previous study done with *X. fastidiosa* and other common riparian plants (Purcell and Saunders 1999). After inoculation each stem was evaluated to determine the survival of *X. fastidiosa* within the Bermuda grass. Evaluations took place 1 week, 3 weeks, 6 weeks, and 9 weeks after inoculations. At each evaluation period, 25 stems were tested. Each stem was tested at two points, one just above the inoculation point before the third node, and between the third and fourth node. Each stem was tested for amount of *X. fastidiosa* detected per gram of tissue. Detection of the bacterium was conducted by culture. Methods for culturing, from preparation of the sample, periwinkle wilt-Gelrite (PWG) media, surface sterilization and dilution plating has been previously described (Hill and Purcell 1995).

Transmission by the red-headed sharpshooter Red-headed sharpshooters were placed on an infected grape *Vitis vinifera* (Grape A) for a period of 48 hours (Fig. 1).

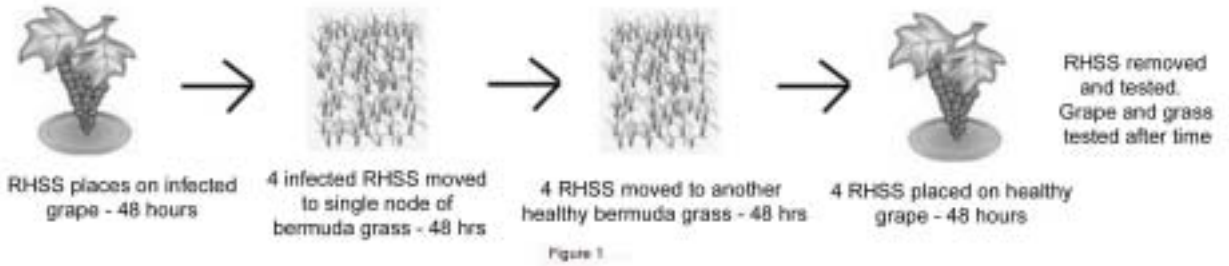

Figure 1

Grape A had been inoculated mechanically by needle and had tested positive for *X. fastidiosa* by culture. The time frame of 48 hours was determined from a previous study (Purcell 1979), which found no significant increase in leafhopper acquisition after 24 hours, thus 48 hours ensured adequate time for acquisition. The insects were then transferred in groups of four to a

single stem of Bermuda grass (BG-1). BG-1 was from the same clone described earlier. The groups of 4 RHSS were allowed to feed for 48 hours. After feeding on BG-1 they were moved in the same groups of fours to another stem of Bermuda grass (BG-2), and again allowed to feed for 48 hours. Finally each group of four redheaded sharpshooters were placed on a healthy non-infected grape *Vitis vinifera* (Grape B). These grapes were propagated from seeds; thus the possibility of infection by the bacteria was minimal. After the removal of the insects, all plants and insects were tested for *X. fastidiosa*. The Bermuda grass was evaluated approximately 3 weeks after insects were taken off, and the grapes were evaluated 30 days after the insects had been removed or when symptoms appeared.

Evaluation of 9 strains of *X. fastidiosa* The survival of nine different strains of *X. fastidiosa* were evaluated in Bermuda grass, designated: Tulare-ALS, Tulare-PD, Medeiros, Conn Creek, Preston, Dixon-ALS, Periwrinkle wilt, STL, and SJV. Bermuda grass stems were planted and inoculated as described earlier. Each strain was inoculated into 20 stems of Bermuda grass, totaling 180 inoculated stems. Based on results from the first part of the experiment, every stem was evaluated at the inoculated node by culture. Three weeks after inoculation 10 stems from each strain were evaluated, totaling 90 stems. After 6 weeks, the rest of the stems were evaluated.

STL Results (Table 1)

1st Week

1st Node CFU/G	2nd Node CFU/G
2.30E+04	
5.60E+04	
2.70E+04	
1.50E+03	
7.30E+05	
5.80E+05	
5/25 Cont	22/25 Cont
6/20 Pos	0/3 Pos

3rd Week

1st Node CFU/G	2nd Node CFU/G
1.70E+04	
1.40E+05	
1.40E+03	
7.80E+03	
1.80E+05	
2.30E+03	
1.00E+05	
10/25 Cont	2/25 Cont
7/15 Pos	0/23 Pos

* Pos = Positive
Cont = Contaminated

6th Week

1st Node CFU/G	2nd Node CFU/G
6.80E+03	
9/25 Cont	0/25 Cont
1/16 Pos	0/25 Pos

9th Week

1st Node CFU/G	2nd Node CFU/G
12/25 Cont	2/25 Cont
0/13 Pos	0/23 Pos

Table 1 - Results from positive samples are listed in CFU's / Gram. Contaminated samples were not included into the calculation, but listed here for reference.

Results

Systemic growth in Bermuda grass Of the 50 samples evaluated at each time frame, contaminated samples were not considered. The percentage of samples that were contaminated can be viewed on Table 1.

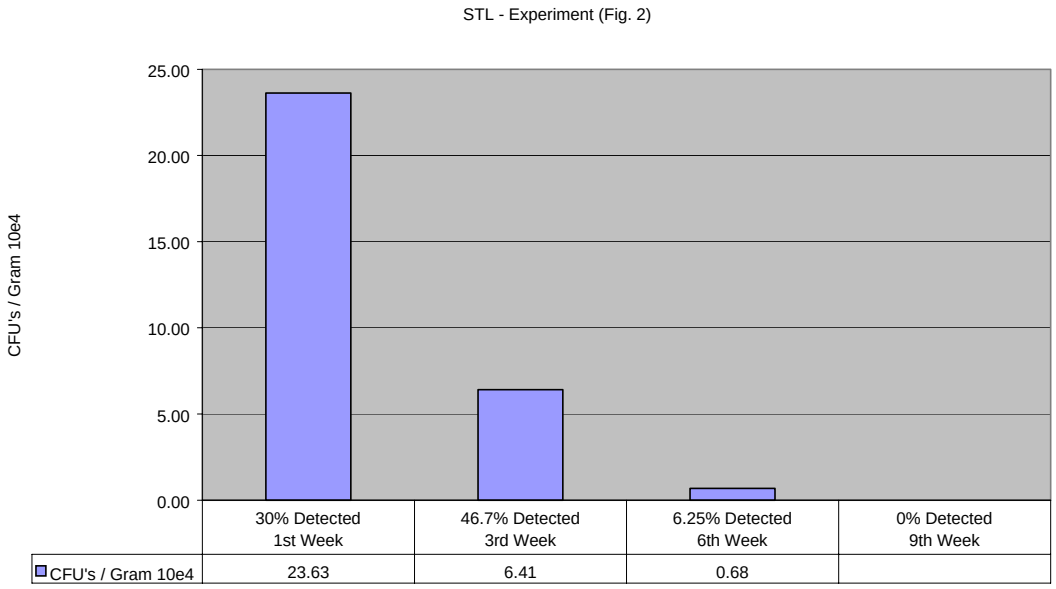

Figure 2 - Averages of CFU/Gram were calculated at each time frame. The percentage of positive samples were also calculated.

Table 1 also displays each evaluation time frame, and CFU/gram of *X. fastidiosa* recovered at each node at that time. At each evaluation the percentage of positive samples, and averages of colony forming units per gram were also calculated (Fig. 2). After one week 6/20 samples tested positive for the bacteria, contaminated samples were not included into the total count. After three weeks 7/15 samples yielded positive data. Finally at the sixth evaluation only one out of 16 samples tested positive for *X. fastidiosa. X. fastidiosa* was never detected in the node preceding the inoculation point, nor after six weeks.

Transmission by the red-headed sharpshooter This experiment was repeated nine times. Four of nine attempts were not included due to insects dying on the acquisition plant (Grape A). The temperature in the insectory may have been too high for RHSS' to survive while feeding on grape plants (~27C). RHSS were moved to a cooler temperature while feeding on grape plants,

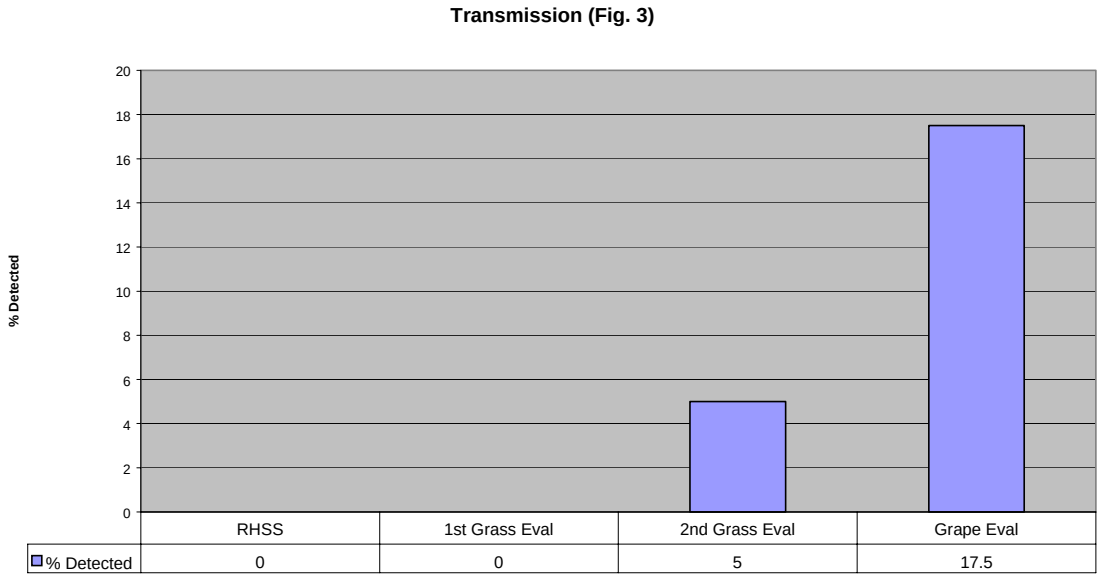

Figure 3 - Displays total percentage of positive detection when evaluated for *Xyella fastidiosa*.

Transmission Experiments (Table 2)

Exp. B

RHSS	1st BG Eval	2nd BG Eval	Grapes
0/29 Cont	9/13 Cont	8/11 Cont	0/8 Cont
0/29 Pos	0/4 Pos	0/3 Pos	2/8 Pos

Exp. F

RHSS	1st BG Eval	2nd BG Eval	Grapes
0/1 Cont	4/11 Cont	2/11 Cont	0/10 Cont
0/1 Pos	0/7 Pos	0/9 Pos	1/10 Pos

Exp. G

RHSS	1st BG Eval	2nd BG Eval	Grapes
0/22 Cont	12/12 Cont	12/12 Cont	0/12 Cont
0/22 Pos	0/0 Pos	0/0 Pos	3/12 Pos

Exp. H

RHSS	1st BG Eval	2nd BG Eval	Grapes
3/10 Cont	0/4 Cont	1/4 Cont	0/4 Cont
0/7 Pos	0/4 Pos	1/3 Pos	1/4 Pos

Exp. J

RHSS	1st BG Eval	2nd BG Eval	Grapes
0/21 Cont	1/6 Cont	1/6 Cont	0/6 Cont
0/21 Pos	0/5 Pos	0/5 Pos	0/6 Pos

Table 2 - List the successful trials for RHSS transmission. RHSS list the results of the evaluation of RHSS heads. 1st BG and 2nd BG evaluation are the Bermuda grass stems evaluated after 3 weeks. Grapes refers to evaluation of Grape B.

after which the survival rate of the insects increased. The starting number of RHSS varied because the fatality rate on Grape A varied. Of the RHSS heads that were evaluated for *X. fastidiosa*, none were positive (Fig. 3).

Strains Experiment Results (Table 3)

5/9 Positive Strains

Mederios		**Comm Creek**		**Preston**	
3 weeks CFU/G	6 weeks CFU/G	3 weeks CFU/G	6 weeks CFU/G	3 weeks CFU/G	6 weeks CFU/G
8.30E+04	2.30E+05	2.70E+03	2.50E+05	2.80E+04	9.80E+02
6.20E+04	2.30E+03	6.30E+03		1.60E+04	1.30E+05
2.10E+04		5.60E+03		5.80E+03	5.40E+04
3/10 Cont	0/10 Cont	0/10 Cont	5/10 Cont	2/10 Cont	0/10 Cont
3/7 Pos	2/10 Pos	4/10 Pos	1/5 Pos	4/8 Pos	3/10 Pos

STL		**SJV**	
3 weeks CFU/G	6 weeks CFU/G	3 weeks CFU/G	6 weeks CFU/G
2.80E+04		2.20E+04	
1.70E+04			
9.30E+04			
8.10E+04			
9.60E+05			
2.20E+04			
3/10 Cont	4/6 Cont	3/10 Cont	2/10 Cont
6/7 Pos	0/2 Pos	1/7 Pos	0/8 Pos

Table 3 - Results from 5/9 strains inoculated into Bermuda grass. Positive samples

All samples of BG-1 were also negative by culture. One BG-2 sample was positive as well as several from Grape B (Table 2). Table 2 displays the five trials that were successful. The RHSS survival rate was not constant through out the transmission process. The number of RHSS present fluctuated between each transmission point, depending on survival rate. The results of total number of RHSS at the end of each trial are shown on Table 2. Each set of RHSS were enclosed in cages, the healthy grapes had no contact with any other insects than the ones they were designated for. With no outside influence, detection of *X. fastidiosa* in the grapes strongly suggest RHSS inoculation.

Evaluation of 9 strains of *X. fastidiosa* Figure 4 and Figure 5 display the results of positive samples from weeks 3 and 6 respectively. At 3 weeks, five of nine of the strains were detected in Bermuda grass stems at the inoculated node (Medeiros, Comm Creek, Preston, STL, SJV). After 6 weeks, only 3/9 were detected (Comm Creek, Mederios, Preston). 4/9 strains were not recovered at either 3 weeks or at 6 weeks (Tulare-ALS, PD, Dixon-ALS, Peri-wrinkle wilt).

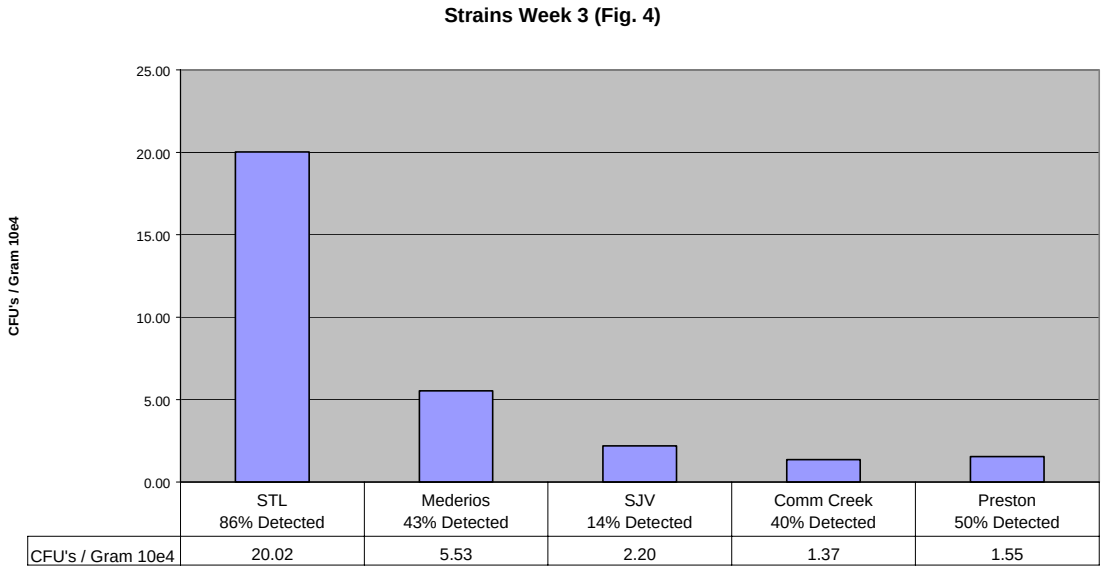

Figure 4 - Week 3. Averages of CFU's / Gram for each positively detected strain. Also displays percentage of positively detected samples.

Again contaminated samples were not included when calculating percent recovered. Numbers of contaminated and non-contaminated are given in Table 3.

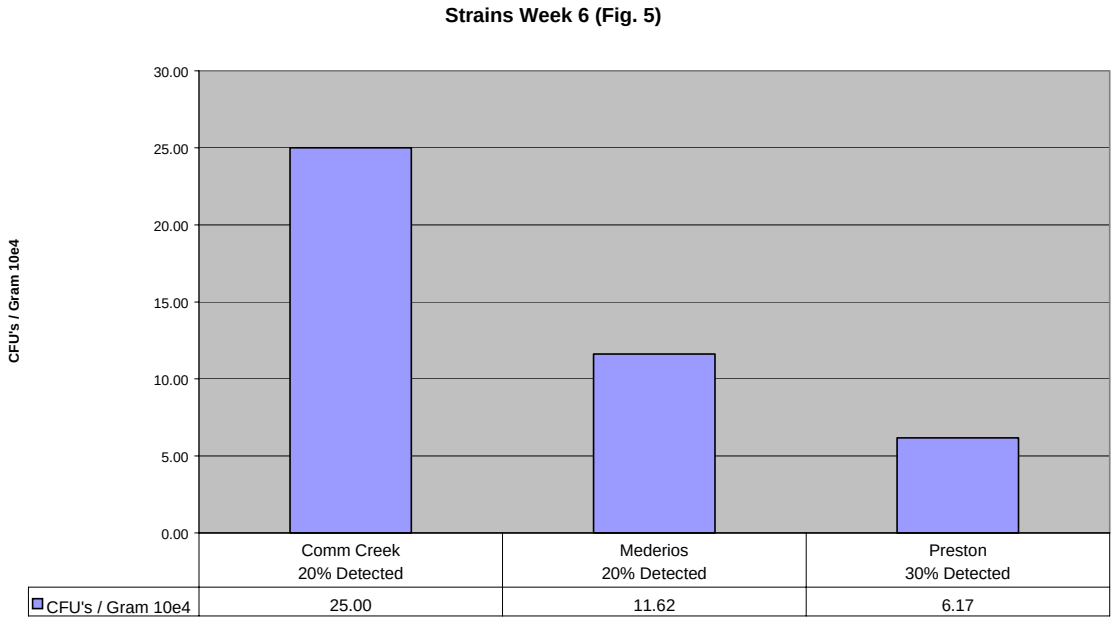

Figure 5 - Week 6. Averages of CFU's / Gram for each positively detected strain. Also displays percentage of positively detected samples.

Discussion

Results from the first part of this experiment suggest that *X. fastidiosa* can survive and multiply in Bermuda grass at least up to 6 weeks. Results also suggest that *X. fastidiosa* is not systemic in Bermuda grass, but rather survival is limited to the infected node. The method of detection utilized in this experiment has a 5% random probability of not detecting one or more CFU if the sample has a population of 300 CFU/g (Hill and Purcell 1995b). *X. fastidiosa* may have resided in the second node of the Bermuda grass stem, yet could not be detected by culture. *X. fastidiosa* may have also been present in some samples, yet could not be recovered due to contaminants. The relative frequencies of positive samples in the first and second week are inaccurate, due to contamination. Despite this, the positive recovery of *X. fastidiosa* indicates sustainable growth of the bacteria up to a time before nine weeks.

The next step of the experiment was to determine the efficiency of the RHSS to recover and transmit the bacterium. No RHSS evaluated tested positive for *X. fastidiosa*. Again the amount of bacterium present in the leafhopper may not have been large enough to be detected by culture. Only small numbers of *X. fastidiosa* in vector's heads are needed for efficient transmission to plants (Hill and Purcell 1995a). This appears to be a reasonable statement, because *X. fastidiosa* was in fact recovered from grape plants that had been inoculated by the RHSS.

Five out of 9 strains tested were detected after three weeks, indicating that multiple strains of *X. fastidiosa* could survive in Bermuda grass. Although Bermuda grass may not be a systemic host, it appears to have the ability to harbor *X. fastidiosa* long enough for transmission. Although no studies to date have tested the exact duration needed for the RHSS to acquire the bacteria from Bermuda grass, previous studies were conducted with similar leafhoppers. The leafhopper, *Graphocephala atropunctata*, has the ability to acquire and transmit *X. fastidiosa* from infected grape within only 2 hours (Purcell 1979). RHSS feeding on Bermuda grass or grape could have the potential to acquire the bacterium and transmit it to another plant. This poses a serious threat because in certain areas, Bermuda grass grows near-by vineyards, and harbor a large population of red-headed sharpshooters. The nearby vineyards display symptoms of Pierce's disease (Purcell 2000, pers. comm). The importance of Bermuda grass as a potential host of *X. fastidiosa* lies in its ability to harbor high numbers of redheaded sharpshooters, and its fast growth under unfavorable conditions. With this combination there is definitely potential for spread and retention of the bacterium. Yet given the findings in this experiment, Bermuda grass

does not seem an efficient host for the bacteria. Since the bacteria was not detected after 9 weeks, transmission would most likely have to occur within 6 weeks after inoculation. Also acquisition of the bacteria would most likely have to occur in the inoculated node. With these factors considered, Bermuda grass and the RHSS do have the potential for the spread of Pierce's disease, but with low probablity.

Ecological studies can follow this experiment to determine how the proximity of Bermuda grass to vineyards will effect the spread of PD. Utalizing another method of detecting *X. fastidiosa* below the 300 CFU/gram threshold, may provide additional clues on the impact Bermuda grass has on the spread of Pierce's disease.

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