EFFECTS OF FEEDING SUBSTRATE ON RETENTION AND TRANSMISSION OF *XYLELLA FASTIDIOSA* STRAINS BY THE GLASSY-WINGED SHARPSHOOTER

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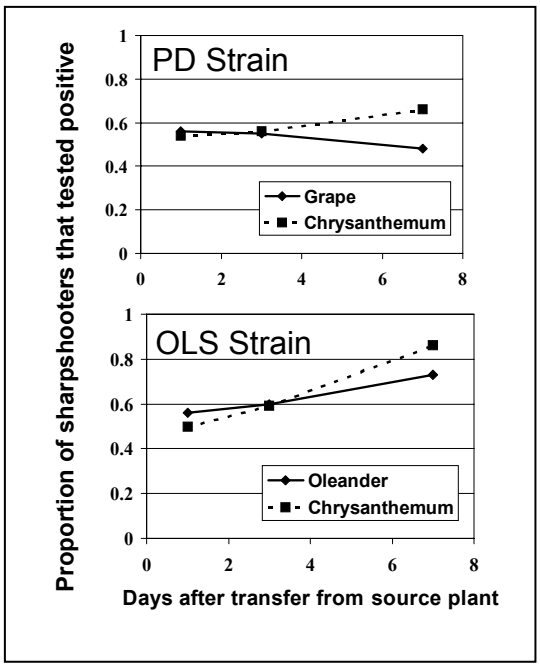
ABSTRACT
In this project we are testing the effects of feeding substrate on the acquisition and retention of *Xylella fastidiosa* by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*. We are using two strains of *X. fastidiosa* that are present in California: a Pierce's disease (PD) strain that infects grape, and an oleander leaf scorch (OLS) strain that infects oleander. A series of experiments were conducted to compare the retention of PD or OLS strains after acquisition, when insects were subsequently maintained on a plant species that was either a host or non-host of that particular strain. In these studies, we found no significant difference in the mean proportion of insects testing positive for the PD or OLS strains, regardless of whether the insects were subsequently fed on either a host or a non-host of the PD or OLS strain. Thus, retention of a particular strain of the pathogen by an individual insect does not appear to be dependant on the xylem content of the plant host on which it is feeding. In a second study transmission efficiency of adult GWSS fed for 24 h on *X. fastidiosa*-infected plants was compared to those fed for 24 h on *X. fastidiosa* from pure media-grown cultures delivered through a cut stem system. In these experiments insects transmitted PD and OLS strains when they acquired the bacteria from a plant, but did not transmit either strain when media-grown bacteria were delivered through the cut-stem system.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is capable of acquiring and transmitting several different strains of *X. fastidiosa* from a variety of host plants. In this project we are testing the effects of feeding substrate on the acquisition, retention and transmission of *X. fastidiosa* by GWSS. Two strains of the pathogen present in California are being used in these experiments: a Pierce's disease (PD) strain that infects grapevine, and an oleander leaf scorch (OLS) strain that infects oleander. These two strains have different host ranges; the PD strain does not infect oleander, and the OLS strain does not infect grape.

OBJECTIVES
1. Compare retention times of *X. fastidiosa* when infected glassy-winged sharpshooter (GWSS) are subsequently fed on plants that are either hosts or non-hosts of the strain they carry.
2. Compare acquisition and transmission efficiency of insects fed on infected plants to those fed on media-grown cultures delivered through cut stems.
3. Compare retention times of two strains of *X. fastidiosa* in GWSS when simultaneously acquired through cut stems, then subsequently fed on either (a) a non-host of both strains, (b) on a host of only one strain, or (c) alternating hosts of each strain.
4. Test the effects of antibacterial materials on acquisition and transmission of *X. fastidiosa* by GWSS.
5. Test the effects of variation in substrate pH and free ion availability on the acquisition and transmission of *X. fastidiosa* by GWSS.

RESULTS
Objective 1
We began by comparing the relative proportion of insects that tested positive after acquisition of a given strain of *X. fastidiosa,* when they were subsequently maintained on a plant species that was either a host or non-host of that strain. Grape plants (*Vitis* spp.) infected with a Pierce's disease (PD) strain of *Xylella fastidiosa*, and oleander plants *(Nerium oleander*) infected with an oleander leaf scorch (OLS) strain were used as sources of inoculum. The strain of *X. fastidiosa* infecting plants was confirmed by PCR. Groups of GWSS adults were caged on either an OLS infected oleander plant, or a PD infected grapevine for 2 days. Insects were then moved to an uninfected plant of the same species as the source plant (oleander or grape), or to a non-host of the strain (chrysanthemum). Samples of insects were collected at 1, 3, and 7 days after transfer to uninfected hosts and frozen. Insects were subsequently tested for the presence of *X. fastidiosa* using PCR.



Results from retention experiments using the OLS strain acquired from oleander showed no significant difference in the mean proportion of insects testing positive when insects were subsequently fed for on either a host (oleander), or a non-host (chrysanthemum) of the OLS strain for 1, 3, or 7 days after acquisition. Similarly experiments using the PD strain acquired from grapevine also found no significant difference in the mean proportion of insects testing positive at 1, 3 or 7 d after acquisition regardless of whether the insects were subsequently fed on either a host (grapevine) or a non-host (chrysanthemum) of the PD strain. Thus, both PD and OLS strains of *X. fastidiosa* remained detectable in GWSS, even when the insects fed on a non-host of the strain for 7 d.

Objectives 2 and 3

To test if feeding substrate can influence the ability of insects to acquire and transmit a particular strain of *X. fastidiosa*, we plan to use a pathogen delivery system to allow us to either maintain or manipulate the feeding substrate as desired. The method described by Bextine and Miller (2002) was originally used for an *Alcaligenes* sp. of bacteria. This technique was modified to provide an environment suitable to survival of *Xylella fastidiosa* and the test plants used. We are using sections of chrysanthemum stem about 10 cm long that are connected by tubing to a syringe with a suspension of *X. fastidiosa* in PBS. The distal end of the stem is also cut and left open. The syringe is depressed until liquid is extruded from the distal end of the cut stem. Then GWSS are allowed to feed on these stems.

To demonstrate that live *X. fastidiosa* cells could survive movement through a cut stem, *X. fastidiosa* was suspended in a PBS buffer, and the syringe was depressed until liquid was extruded from the distal end of the cut stem. Droplets forming on the distal end were collected and analyzed using PCR to determine if *X. fastidiosa* cells were present. In all cases, *Xylella* was detected within the first 10 drops extruded. Thus, in these experiments, material was injected into stems until at least 10 drops of material was extruded from the distal cut end to ensure that the bacteria have been moved the entire distance of the stem.

In transmission experiments, adult insects were fed for 24 hours on either infected plants, or media-grown bacteria delivered through the cut stem system as described above. Adults were then individually moved to uninfected test plants and allowed to feed for 4 d. When GWSS adults were fed on PD-infected grapevines, 12/26 (46%) transmitted the pathogen to healthy grapevine test plants. In contrast, when insects were fed on media-grown PD bacteria through the cut stem method, no individuals (0/48) transmitted the pathogen to test pants. Similar results were found with OLS-infected plants (9/37,or 24% of individuals transmitted) compared to media-grown OLS delivered through cut stems (0/22 transmitted). Thus, insects did not transmit PD or OLS strains when media-grown bacteria were delivered through the cut-stem system. Purcell et al. (personal communication) found similar results when leafhoppers were fed *X. fastidiosa* through parafilm sachets.

Additional studies are being conducted to determine why insects are unable to transmit the pathogen from the cut stem delivery system. For example, a recent study demonstrated that *X. fastidiosa* cultures will produce different levels of "biofilm formation" when grown on different types of media (Leite et al. 2004). We will test if growing our strains on different media may help induce transmissibility by insects. In addition, we will conduct further studies to determine the pathway of the bacteria through the system. For example, by testing the honeydew of insects feeding on the cut stem system, we can determine if the bacteria are successfully passing through the insect. In the interim, work on the remaining objectives will continue using insects fed on PD and OLS infected plants.

CONCLUSIONS

In retention experiments (Objective 1) for both the PD and OLS strains, we found the proportion of insects retaining the pathogen was the same, regardless of whether insects subsequently fed on a host or a non-host of that strain. This indicates that retention of a particular strain of the pathogen by an individual insect is not dependant on host-specific xylem content of the plant on which it is feeding. In transmission experiments (Objectives 2 and 3) insects successfully transmitted the PD and OLS when they acquired the pathogen from infected grapevine and oleander plants respectively, but did not transmit either the PD or OLS strains when the media-grown bacteria were delivered through the cut-stem system. This could be the result of biological characteristics of media-grown bacteria that contribute to non-transmissibility by insects, or failure of the cut stem system to properly deliver bacteria to the insect. Further experiments are being conducted to determine the basis for lack of transmission of media-grown bacteria by GWSS.

REFERENCES

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