SYMBIOTIC CONTROL OF PIERCE'S DISEASE: TESTING REAGENTS AGAINST *XYLELLA FASTIDIOSA*

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ABSTRACT
Pierce's disease (PD) is caused by a xylem limited gram-negative *Xylella fastidiosa* (*Xf*) bacterium. Various species of sharpshooters, including the important glassy-winged sharpshooter (GWSS), transmit *Xf*. Currently, there is no cure for PD. Paratransgenesis is a new tool for the management of PD. Acquisition efficiency of GWSS to acquire *Xf* is about 80% when tested with Real-Time PCR. Results of selected phage antibody specific to *Xf* PD-strain to disrupt the pathogen are underway.

INTRODUCTION
Strains of *Xylella fastidiosa (Xf)*, a gram-negative bacterium, cause a number of important plant diseases including Pierce's disease (PD) in grapevine, citrus variegated chlorosis (CVC) in citrus, phoney peach disease, periwinkle wilt, and leaf scorch disease in plum, elm, maple, sycamore, and coffee (Hopkins 1989).

The principal vector for the transmission of *X. fastidiosia* is the glassy-winged sharpshooter (GWSS) (*Homalodisca coagulata*). The pathogen attaches to the cibarium and precibarium of sharpshooters by means of an extracellular matrix (ECM) and is transmitted from infected plants to healthy plants when the sharpshooters feed (Brlansky et al. 1983).

Symbiotic control identifies a symbiont that is genetically modified to produce a gene product that inhibits transmission of a pathogen. Recent examples of symbiotic control are the control of Chagas' disease caused by *Trypanosoma cruzi* and transmitted by the Triatomid bug *Rhodnius prolixus* (Durvasula et al. 1997), the prevention of Colitis in mammals (Beninati et al. 2000, Steidler et al. 2001), and to interfere with HIV transmission (Chang et al. 2003).

This approach is being developed for the management of PD. *Alcaligenes xylosoxidans* subsp. *denitrificans* (*Axd*) was chosen for genetic modification to deliver an anti-*Xylella* product. *Axd* is appropriate because it shares the same niche as *Xf* in the foregut of the GWSS and cycles well between the insect and plant system. Also, this bacterium has been described as a non-pathogenic soil-borne microbe and a non-pathogenic endophyte (Meade et al. 2001).

Single chain antibody (scFV S1), which is expressed on the surface of a M13 bacteriophage, has been selected against *Xf* PD-strains by using a panning technique. S1 is supposed to bind to the surface of a *Xf* PD-strain. Currently we are testing S1 in an *in vitro* insect-plant-pathogen system.

OBJECTIVES
1. Test the acquisition of *Xf* by GWSS feeding on infected *Vinca major*.
2. Test the efficiency of S1 to inhibit *Xf* transmission on *V. major*.

RESULTS
Field collected GWSS from a citrus orchard were put into an artificial feeding system (AFS) to acquire S1. Afterwards the GWSS were allowed an acquisition access period (AAP) on a *Xf* PD-strain infected *V. major* for 48 hours. Then, these sharpshooters were transferred onto clean test *V. major* plants and allowed an inoculation access period (IAP) of 48 hours. After 6 weeks these test plants were tested for *Xf* colonization by Real-Time PCR (rt-PCR). Negative controls were an anti-BSA phage and PBS. Each of the three treatment groups was mixed with 0.2% dextrose in a 1:4 ratio, respectively. The AFS consists of multiple plastic vials each with a vinyl tube both closed with wrapped parafilm and filled with the appropriate above said solution.

In another set of experiments the field collected GWSS were allowed an AAP of 48 hours on the *Xf* PD-strain infected *V. major*. Then these GWSS were transferred to the AFS to acquire S1, anti-BSA phage, and PBS solution for 48 hours.

Thereafter these GWSS were allowed an IAP on clean *V. major* for 48 hours. And then these plants were tested for *Xf* colonization via rt-PCR after 6 weeks.

In both sets of experiments the transmission of *Xf* by GWSS was tested by allowing the sharpshooters to feed first on the *Xf* PD-strain infected *V. major* for 48 hours. Then these test insects were transferred onto clean test plants to feed for 48 hours. The results of the experiments are pending.

Samples of the GWSS that fed on the *Xf* PD-strain infected plant for 48 hours were taken and then their heads were tested for the presence of *Xf* via rt-PCR. Eighty percent (range 70-100%) of GWSS heads shows the presence of *Xf*. The field collected GWSS were also tested for the presence of *Xf* via rt-PCR. Only 0-10% (mean = 5%) of the field collected GWSS were found to be infected with *Xf*.

CONCLUSION

An effective AFS has been developed to allow the GWSS to acquire S1. *V. major* was selected as the model plant for our insect-plant-pathogen system to test S1. Eighty percent of the GWSSs acquire *Xf* after 48 hours of AAP. Experiments on the disruption of *Xf* by S1 are ongoing.

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