# ENVIRONMENTAL FATE OF A GENETICALLY MARKED ENDOPHYTE IN GRAPEVINES AND THE GLASSY-WINGED SHARPSHOOTER

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**Reporting Period:** The results reported here are from work conducted from July 1, 2003 to October 31, 2003.

## ABSTRACT
A permit was obtained from EPA in late spring to conduct field trials in covered grapevines at three commercial vineyards and one experiment station in California. *Alcaligenes xylosoxidans denitrificans* (*Axd*) bacteria, modified to produce a fluorescent protein, were applied to grapevines by needle inoculation, foliar spray application, and soil drench. The plants were covered with insect-free screening, to exclude arthropods from test plants. Samples were taken throughout the growing season and are currently being processed. Grapevines at the Riverside field site were exposed to glassy-winged sharpshooters to test the affect of insect feeding on the translocation of *Axd* in grapevines. Samples from these plots are being analyzed. The results are too preliminary to report at this meeting.

## INTRODUCTION
Paratransgenesis employs symbiotic bacteria to deliver anti-disease compounds to target pathogens of plants to make vector insects unable to harbor the pathogen or to prevent a pathogen from being transmitted to healthy plants. *Alcaligenes xylosoxidans denitrificans* (*Axd*) was selected for further study and genetically altered with a fluorescent marker. We propose to follow the movement of *Axd* in grapevines and in the vector insect, the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*.

Regulatory and industry confidence in this approach require knowing the fate of *Axd* in various locations, various plant types and the spread at different locations and in plants at different times of the year. Our current detection methods employ PCR (polymerase chain reaction) and fluorescence microscopy. RT-PCR provides a quantitative measure of bacteria in the samples, which is missing from existing methods. This is important because it relates to determining optimum dose and timing for application and expression of the anti-*Xylella* compound.

Fluorescent protein gene markers are now commonly used in genetics and are not considered an environmental danger since they are based on natural compounds. The bacterial transformation cassette was inserted with so-called jumping genes (mobile or transposable elements) originally identified in *Drosophila mauritiana* and called mariners. The mariner elements have had their jump mechanism removed (so the inserted gene will not be mobilized) and all antibiotic genes used for selection have been removed (so no antibiotic factors can be moved inadvertently to other bacteria). Our results confirm that the transgenic strains are very stable and grow readily in culture.

Since the marker genes were placed next to an open reading site that is designed to contain the future anti-*Xylella* compound, the bacteria we are using now are nearly complete. In other words it is as close to the final product as we can get without actually using the compound itself. Thus, we can study the biology of the vehicle bacterium, *Axd*, and its behavior in the vineyard ecosystem.

We prefer to do this in vineyards because we feel that laboratory experiments will not be subject to natural forces that will be present in actual crops, and therefore might not be fully indicative of natural fate. It will also be important to choose widely separate locations across the grape growing regions in California for similar reasons. We need to determine now if the transgenic endophyte will travel to the fruit of the grape plant and to learn of its fate during harvesting operations. Our intention is to design a control method that will be considered safe to consumers. Greenhouse experiments will not provide rigorous enough conditions. Therefore it is necessary to obtain field data.

**OBJECTIVES**

1. Track the movement of *Alcaligenes xylosoxidans* (*Axd*) within plants with or without insect involvement and track movement in the environment.
2. Characterize transmission of *Axd* by glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*).
3. Develop an application method for transgenic *Axd* into the xylem of grape plants for delivery of an anti-*Xylella* strategy.

**RESULTS AND CONCLUSIONS**

In July, field sites were established at four locations in the state of California: Napa, Bakersfield, Temecula, and Riverside. At the Napa, Bakersfield, and Temecula sites, *Axd* was applied to grapevines using three inoculation techniques; needle inoculation, foliar spray application, and soil drench. These plants were covered with insect-free screening, to exclude arthropods from test plants. Samples were taken throughout the growing season and are currently being processed. Grapevines at the Riverside field site were needle inoculated with *Axd* and three concentrations of GWSS (0, 10, and 50) were placed on the plants to test the affect of GWSS feeding pressure on the translocation of *Axd* in grapevines. We collected mature grapes and plant parts for analysis from grapevines at all four field sites. We are analyzing whole grapes as well as are dissecting out and surface sterilizing different tissues of the grapes to determine if *Axd* colonizes these tissues. Preliminary results suggest the *Axd* is not present in the fruit of the grape, but samples have not been fully analyzed yet.

**REFERENCES**


Agresti, Alan. 1990. Categorical data analysis. New York, Wiley.

Bell, C.R., G.A. Dickie, W.L.G. Harvey, and J.W.Y.F. Chan. 1995. Endophytic bacteria in grapevine. Canadian Journal of Microbiology 41: 46-53.

Bextine, B.R., A. Wayadande, B.D. Bruton, S.D. Pair, F. Mitchell, and J. Fletcher. 2001. Effect of insect exclusion on the incidence of yellow vine disease and of the associated bacterium in squash. Plant Disease 85: 875-878.

Mansour, N.S. 1984. Floating row covers give plants tlc. American vegetable grower: 8-10.

Natwick, E.T., and F.F. Laemmlen. 1993. Protection from phytophagous insects and virus vectors in honeydew melons using row covers. Florida entomologist 76: 120-126.

Nault, L.R. 1997. Arthropod transmission of plant viruses: a new synthesis. Ann. Entomol. Soc. Am. 90: 521-541.

Oliveira, A.C., M.A. Vallim, C.P. Semighini, W.L. Araujo, G.H. Goldman, and M.A. Machado. 2002. Quantification of *Xylella fastidiosa* from citrus trees by real-time polymerase chain reaction assay. Phytopathology 92: 1048-1054.

Perring, T.M., R.N. Royalty, et al. 1989. Floating row covers for the exclusion of virus vectors and the effect on disease incidence and yield of cantaloupe. Journal of economic entomology 82: 1709-1715.

Purcell, A.H., and A Finlay. 1979. Evidence for noncirculative transmission of Pierces disease bacterium by sharpshooter leafhoppers. Phytopathology 69: 393-395.

Purcell, A.H. 1997. *Xylella fastidiosa*, a regional problem or global threat? Journal of Plant Pathology 79: 99-105.


**FUNDING AGENCIES**


Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.