

# RAPID BIOLUMINESCENT MONITORING OF INFECTION BY THE PIERCE'S DISEASE AGENT

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**ABSTRACT**

This report summarizes the goal of a new project focused on bioluminescent quantification and imaging as an approach to monitor *Xylella fastidiosa* infection of grapevine. This research will be applied to addressing how *X. fastidiosa* grows within and spreads through xylem tissue of infected plants.

**INTRODUCTION**

*Xylella fastidiosa* has a sophisticated biphasic lifestyle that defines the Pierce's disease infectious cycle; it lives exclusively within the xylem tissue of susceptible plants and in the foregut of insect vectors (2, 3). The complex nature of bacterial-host interactions that take place during the Pierce's disease infectious cycle precludes a standard dogmatic approach to rapidly investigate preventative measures to control this devastating disease. Current approaches toward understanding the progression of Pierce's disease are limited by the length of time required to evaluate disease progression. Symptoms of Pierce's disease often appear only after many weeks or months of grapevine infection with *X. fastidiosa* (2, 3). At earlier stages of infection, *in planta* observations of infection are limited to procedures that are quite time consuming, often are destructive and necessitate large sample sizes to correlate experimental observations with disease pathology. Our goal is to develop a new approach to probe host-pathogen interactions that take place during Pierce's disease of grapevines.

Procedures currently used to detect *X. fastidiosa* infection of grapevine include bacteriological isolation of bacteria from infected plant tissue, the detection of bacterial antigens in plant tissue by ELISA and detection of *X. fastidiosa* nucleic acids by PCR. Each of these techniques provides valuable information, but are limited because they are somewhat expensive, time consuming and require significant numbers of samples to provide statistically significant results. The goal of this study is to develop bioluminescent techniques to extend the current limits of Pierce's disease investigation. The use of bioluminescent technology can overcome many of the current experimental limitations faced by researchers and has some additional benefits. First, strains of *X. fastidiosa* engineered to be bioluminescent can be monitored directly and their numbers can be quantified with the use of a luminometer or a photon sensitive camera coupled to a computer. This time-efficient and cost-effective method of monitoring *X. fastidiosa* infection of grapevines will provide valuable information about the progression of Pierce's disease at pre-symptomatic stages of infection and may reduce the number of plants that need to be infected to complete an experiment. Second, the use of bioluminescent strains of *X. fastidiosa* may allow quantification of growth and spread of bacteria in host tissues. We will evaluate the potential use of *in vivo* bioluminescent imaging (IBI) of whole plants as a non-destructive approach to monitor *X. fastidiosa* growth and spread during infection of grapes. IBI has previously been used to monitor cellular activities in plants and is a proven concept that has already been used by plant physiologists (1, 4). In this project, we present the application as a novel approach to analyze Pierce's disease. When used to its full potential, IBI represents a nondestructive approach that can provide insight at multiple time points over the course of hours, days and weeks from the point of *X. fastidiosa* entry into the plant through systemic spread of the bacteria in xylem tissue. If successful, IBI can be further exploited to rapidly evaluate protective methods and intervention procedures to limit Pierce's disease. In addition, this experimental approach may allow us to rapidly evaluate current and newly developed cultivars of grapes for resistance to *X. fastidiosa* infection.

IBI measures light emitted by specially engineered strains of *X. fastidiosa* from sources within living tissues. The emission of light by the bacteria does not cause any deleterious effects to biological samples (1). Thus bioluminescent strains of *X. fastidiosa* offer us the opportunity to take a novel approach that will impact our understanding of Pierce's disease. The success of this research project should help expedite the treatment discovery and development process, thereby offering partners vested in the Pierce's Disease Program the potential to save both time and money in taking prevention strategies into the field. In addition, IBI provides sensitive spatial resolution of the location of infecting bacteria within the plant providing insight on the development of plant infection not achievable with other approaches. Imaging of bacterial infections within living plants provides the opportunity to further our understanding of the processes leading to symptoms of Pierce's disease.

## OBJECTIVES

The primary goal of this research project is to use bioluminescent quantification and imaging as an approach to monitor *Xylella fastidiosa* infection of grapevine. This research will be applied to addressing how *X. fastidiosa* grows within and spreads through xylem tissue of infected plants. More specifically, this approach will allow us to evaluate the hypothesis that *X. fastidiosa* growth within xylem tissue coincides with the restriction of xylem fluid transport.

1. Engineer virulent strains of *X. fastidiosa* that produce luciferase.
2. Monitor bioluminescence of Lux<sup>+</sup> strains of *X. fastidiosa* during *in vitro* growth in a defined medium and xylem fluid.
3. Examine growth and spread of bioluminescent *X. fastidiosa* during infection of grapevines.

## RESULTS AND CONCLUSIONS

This is a new project that received funding in October of 2003. Results from this project will be available during this next year.

## REFERENCES

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4. Scheel, D., and N. Nass. 2001. Enhanced luciferin entry causes wound-induced light emission in plants expressing high levels of luciferase. *Planta* 212: 149-154.

## FUNDING AGENCIES

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***Section 2B:***  
***Glassy-winged Sharpshooter***  
***Biology, Including Plant-***  
***Insect Interactions***

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