

Renewal Progress Report for CDFA Agreement Number 09-0781

Project Title. Field evaluation of grape plants expressing potential protective DNA sequences effective against Pierce's Disease.

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Reporting Period: The results reported here are from work conducted July 1, 2011 to March 3, 2012

INTRODUCTION

The objective is to evaluate transgenic grape and grape rootstocks expressing various genes from different constructs in a field site in Solano County for resistance to *Xylella fastidiosa* (Pierce's Disease strain) following mechanical injections of *X. fastidiosa* into the plant stems. Over the course of the 3 year field evaluation, test plants will include ungrafted conventional Thompson Seedless and Freedom plants as controls, transgenic plants from Dandekar, Labavitch, Lindow and Gilchrist projects and, as plant material availability permits, transgenic rootstocks expressing some of the test genes grafted to untransformed PD susceptible scions will be introduced. All plants were moved as vegetative material in 2010 and 2011 to the APHIS-approved field area with no risk of pollen or seed dispersal and stored on-site in lath houses until planted. The area is adjacent to experimental grape plantings that have been infected with Pierce's Disease for the past two decades following mechanical inoculation in a disease nursery near this site. Over this period there has been no evidence of spread of the bacteria to uninfected susceptible grape plantings adjacent to the infected plants. In addition, there are 500 grape plants that were inoculated and infected with Pierce's Disease 6 years ago as part of ongoing disease research by another investigator and funded by the Pierce's Disease Research Board. The *X. fastidiosa* in this latter ongoing experiment has not spread to the uninoculated experimental controls within the experiment or to any adjacent experimental grape plants over the past 6 years.

OBJECTIVES

Prepare land area and manage the experimental plants with the following specifications:

- i. Area will be sufficient to accommodate up to 500 plants from Lindow, Gilchrist, Labavitch and Dandekar.
- ii. Row spacing is 15 feet between rows with 4 feet between plants. This spacing requires 18 rows of 28 plants each and includes a 50 foot open space around the planted area. Total area occupied by plants and buffer zones will be a minimum of 1.8 acres.

- iii. Each row will be staked with 7 foot grape stakes supporting 13 gauge wire in two wire trellis system with a stake at each plant site. Wires will be stretched and anchored by 7 foot pressure treated posts at the end of each row.
- iv. Area will be fenced to protect against rabbit invasion. The plants will be irrigated by surface furrow with several pre-plant irrigations to bring the soil to field capacity at the time of plant.
- v. Field crews will be provided from these funds to assist in planting and weeding.
- vi. Irrigation and pest management will be provided by Cooperator Tom Kominek.
- vii. All flowers will be removed and all prunings will be autoclaved before disposal under conditions of the permit.
- viii. Plants will be mechanically inoculated with *Xylella fastidiosa* in 2011 and subsequent years.

RESULTS AND DISCUSSION

All of the above objectives set out for the establishment of this field planting were completed. Land preparation, fencing, irrigation, planting and weed control were all accomplished in a timely manner to meet the initial planting date of July 12, 2010. The second phase of the planting, including grafted transgenics was completed May 17, 2011.

The 2010 and 2011 plantings of all four investigators survived the winter without loss. The attachment of new shoots to the trellis system, cultivation, and irrigation management progressed in a normal and effective manner (Figure 1). All flowers were removed before opening and extensive pruning was done to manage the plants in a fashion compatible with mechanical inoculation. All flowers and prunings were collected, bagged, and autoclaved before disposal.

As of July 21, 2011, the initial planting and the second 2011 planting individuals are healthy, growing normally and all plants have a normal phenotype, true to the untransformed control plants of each parental genotype (Figure 1). Inoculations of the 2010 plants occurred on July 12 and July 21 (Figures 2). The field planting will provide important data on the effectiveness of any of the transgenic strategies employed by the respective researchers.

Publications: None

Presentations. None

Research relevance. The objective is to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 genes in a field site at UC Davis for resistance to *Xylella fastidiosa* (Pierce's Disease strain) following mechanical inoculation and in a field site at UC Riverside which has endemic sharpshooters carrying Xf. In four inoculation experiments in a controlled greenhouse over a two year period, involving more than 300 transgenic plants of PR1 and UT456, the suppression of PD symptoms and reduction in bacterial titer has been consistent. A positive correlation between the PR1 and UT456 message level, suppression of bacterial titer and absence of PD symptoms was established using qPCR to measure both the message and the bacteria titer. The Solano field experiment will be conducted in two phases. The first phase starting in 2010 will evaluate clonal copies of the fully transformed ungrafted plants that exhibited suppressed PD symptoms and low bacterial titers. The second phase will begin with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks. Over the course of the 3 year field evaluation, test plants in the first planting will include ungrafted conventional Thompson Seedless and Freedom plants as controls to be compared with the transformed plants. Controls in the second phase will include, untransformed rootstocks grafted to the untransformed scions to be compared with equivalent combinations expressing the test genes grafted to untransformed PD susceptible scions.

LAYPERSON SUMMARY

The purpose of the field planting is to evaluate transgenic grape and grape rootstocks under natural field conditions for efficiency in providing protection against Pierce's Disease. The site in Solano County will enable controlled inoculation and close monitoring of the host response in terms of symptoms, bacterial behavior, and plant morphology. While no fruit will be produced, assessment of the growth characteristics of the plants, inoculated and non-inoculated will be made. Over the course of the 3 year field evaluation, test plants in the first planting will include ungrafted conventional Thompson Seedless and Freedom plants as controls, transgenic plants from Dandekar, Labavitch, Lindow and Gilchrist projects and, as plant material from the first planting becomes available, transgenic rootstocks expressing some of the test genes will be grafted to untransformed PD susceptible scions to assess potential for disease suppression in an untransformed scion from signals in the transformed rootstocks.

Status of funds. We anticipate that all funds allocated for fiscal year 2011-2012 will be expended by June 30, 2012.

Intellectual property. None envisioned at this point. All IP is being handled from the previous greenhouse studies under tightly controlled conditions with mechanical inoculation.



Figure 1. Plants are healthy, growing normally and all plants have a normal phenotype.



Figure 2 Mechanical inoculation is performed by pushing a needle from the underside of the cane, placing a 20ul drop of Xf bacteria, and withdrawing the needle draws the drop in by negative pressure.