FIELD EVALUATION OF GRAPE PLANTS EXPRESSING POTENTIAL PROTECTIVE DNA SEQUENCES EFFECTIVE AGAINST PIERCE'S DISEASE

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ABSTRACT
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 (Xf)* (Pierce's disease strain) following mechanical injections of *Xf* into the plant stems. Over the course of the three 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. As additional plant material becomes available, transgenic rootstocks expressing some of the test genes grafted to untransformed Pierce's disease susceptible scions. All field operations and the handling of plant material will proceed under an APHIS permit for these materials and over a specified time period.

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 (PD). 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 three 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.

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 (Xf)* (Pierce's disease strain) following mechanical injections of *Xf* 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 Pierce's disease (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 PD 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 PD 6 years ago as part of ongoing disease research by another investigator and funded by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. The *Xf* 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. The same *Xf* strain will be used to inoculate the plants in this experiment.

OBJECTIVES
Prepare land area and manage the experimental plants with the following specifications:
1. Area will be sufficient to accommodate up to 500 plants from Lindow, Gilchrist, Labavitch, and Dandekar.
2. 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.

3. 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.
4. 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.
5. Field crews will be provided from these funds to assist in planting and weeding.
6. Irrigation and pest management will be provided by Cooperator Tom Kominek.
7. All flowers will be removed and all prunings will be autoclaved before disposal under conditions of the permit
8. 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 (**Figure 1**). The second phase of the planting, including grafted transgenics was completed May 17, 2011 (**Figure 2**).

The 2010 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. 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 3**). Inoculations of the 2010 plants occurred on July 12 and July 21 (**Figures 4, 5 and 6**). The field planting will provide important data on the effectiveness of any of the transgenic strategies employed by the respective researchers.

FUNDING AGENCIES

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

Figure 1. This image illustrates the field preparation, trellis and staking arrangement and a portion of the initial planting at the Solano County site.

Figure 2. This shows the Solano County site as of July 13, 2011 with newly planted grafted plants in the front rows and the Fall 2010 planting in the back rows.

Figure 3. Plants are healthy, growing normally and all plants have a normal phenotype.

Figure 4. Inoculation of grape vines with *Xf* at the Solano County site is a two or three person task.

Figure 5. 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.

Figure 6. Inoculated grape vine canes were marked with orange tags at the Solano County site.