Interim Progress Report for CDFA Agreement Number 12-0444-SA

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

Principal Investigator	David Gilchrist	Department of Plant Pathology, UC Davis	dggilchrist@ucdavis.edu
Co-Principal Investigator	James Lincoln	Department of Plant Pathology, UC Davis	jelincoln@ucdavis.edu
Collaborator	Thomas Kominek	Department of Plant Pathology, UC Davis	tekominek@ucdavis.edu
Collaborator	Abhaya Dandekar	Department of Plant Sciences, UC Davis	amdandekar@ucdavis.edu,
Collaborator	John Labavitch	Department of Plant Sciences, UC Davis	jmlabavitch@ucdavis.edu
Collaborator	Steven Lindow	Department of Plant and Microbial Biology, UC Berkeley	icelab@berkeley.edu

Reporting Period: The results reported here are from work conducted October 2013 to March 2014

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, 2011, and 2012 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. Hence, there is a documented historical precedent for the lack of spread of the bacteria from inoculated to non-inoculated plants, an important consideration for the experiments carried out for this project.

Objectives

A. Land preparation, planting, and management of the experimental resources to accommodate 500 plants. Plants were introduced with a row spacing of 15 feet between rows and 4 feet between plants in a row. There is a 50 open space buffer area surrounding the field, which is

fenced to protect against rabbits. Each row is staked with 7 foot grape stakes supporting 13 gauge wire in two wire trellis system with a stake at each plant site. Wires are stretched and anchored by 7 foot pressure treated posts at the end of each row. The plants are irrigated by surface furrow in accordance with standard practices for maintaining grapes for experimental purposes at this site. However the plan is to move to drip irrigation in 2014. Irrigation and pest management, primarily powdery mildew and insects, is coordinated or conducted by the Cooperator Tom Kominek, Field Superintendent for the Department of Plant Pathology. Mr. Kominek has 30 years' experience working with grapes under experimental conditions for USDA and UC Davis scientists. He will work closely with PI Gilchrist to determine timing and need of each of the management practices.

B. Principal Investigators with assistance from contract field crews are responsible for pruning in the spring of each year and within the season as needed to maintain a reasonable canopy, given that many of the plants are derived from a rootstock (Freedom) and Thompson Seedless both of which exhibit tremendous vegetative growth during the season. In addition, the pruning will deviate from conventional practice in that additional cordons and season canes will be maintained to enable multiple cane inoculations to provide sufficient inoculated and control material for destructive sampling throughout each season.

C. Irrigation and pest management will be provided by Cooperator Tom Kominek as needed. Irrigation from 2010 to current has been by surface flooding. Plans are in place to install drip irrigation on all plants in summer of 2014.

D. Plants will be mechanically inoculated with *Xylella fastidiosa* by the Investigators beginning in 2011 and subsequent years.

Description of activities conducted to achieve the objectives and progress

All of the above objectives set out for the establishment and management of this field planting were completed in the timelines proposed. 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 and 2). The second phase of the planting, including grafted transgenics was completed May, 2011 and June of 2012.

The 2010 and 2011 plantings of all four investigators survived the winter of 2011 without loss. The attachment of new shoots to the trellis system, cultivation, and irrigation management progressed in a normal and effective manner. Extensive pruning during the season is now recognized as necessary to manage the plants in a fashion to allow ease of mechanical inoculation and recovery of experimental samples.

As of July 21, 2011, the initial 2010 planting and the second 2011 planting individuals are healthy, growing normally and all plants had a normal phenotype, true to the untransformed control plants of each parental genotype. Inoculations of the 2010 plants occurred on July 12 and July 21, 2011. The field planting will provide important data on the effectiveness of any of the transgenic strategies

employed by the respective researchers.

The plants showed limited or no death when emerging in the spring of 2012 (Figure 3). As of July 2012, the inoculated controls and transgenic plants appear healthy and were re-inoculated in June of 2012. By October 2012, some of the control plants were showing symptoms consistent with Pierce's Disease with some inoculated canes dying at this point.

As of July 1, 2013, many inoculated canes on control plants and some transgenics did not survive the winter but the non-inoculated canes on these plants still appear healthy (Figure 4). Visual observation and destructive sampling of inoculated canes indicates that mechanical inoculation was successful in infecting inoculated canes. In addition, the healthy uninoculated canes, adjacent to inoculated canes, suggest that the bacteria have not yet moved systemically through the plants. Plant turgor has been maintained throughout the growing season and there have been no evidence of wilt symptoms prior to death of inoculated control canes.

As of October 2013, the inoculated plants were confirmed to harbor the introduced *Xf*. Uninoculated individuals are healthy, growing normally All plants continue to exhibit a normal phenotype, true to the untransformed control plants of each parental genotype. Uninoculated control plants appear healthy while the inoculated controls and some of the experimental lines are showing symptoms of PD (Figure 5). However, several of the lines from each of the investigators are free of symptoms. **At this stage it is clear that there is a rich source of additional data to be collected from this field experiment that will prove important as we move forward in experiments to combine the best of the transgenes into commercially accepted rootstocks in order to assess trans-graft protection. Field data over the course of this experiment has been collected by all investigators and can be found in their individual reports.**

We propose to continue maintaining and collecting data from this site for the coming 2 years through June 30, 2016. This time period matches the time extension proposed by Dr. Dandekar, who has now assumed responsibility for the APHIS permit. Dr. Gilchrist will continue to manage the field operations at this site. The following budget will cover all the cost of continuation of the experiment and removal of the plants at the end of the experimental period. The APHIS permit specifies that the plants are to be removed, burned on site and the field monitored as fallow for an additional year. There is no cost for monitoring after removal.

Evaluation of all plants on March 17-18, 2014 indicated that Thompson Seedless derived plants had 2-3 leaves while the Freedom plants had only slightly swollen buds. There are now substantial differences between inoculated controls plants vs the uninoculated controls and the plants expressing transgenes.

Details of field operations performed on Solano Pierce's Disease Field experiment from February 2013 to March 2014.

Date: Activity

2/15 Tilled for weed control, grapes just beginning to push

3/15-21 Began pruning, carefully leaving all inoculated/tagged branches, PIs demonstrated procedure to ensure inoculated branches were untouched to the same contract crew that

pruned Deborah Golino's grapes in the same area. All prunings left between the rows to dry, then flail chopped and later rototilled to incorporate.

4/1-2 Pruned additional growth to expose inoculated branches,
4/3 Prunings flail chopped and allowed to dry
4/22 Rototilled to incorporate dry material
4/25-26 Hand hoe weeds within the rows, field crew
5/2 Conclude hand hoe weeds within rows, field crew
5/14 Repaired rabbit fence
5/15-17 Rototilled for weed control
5/20 Pulled up furrows in preparation for surface irrigation
5/21-23 Applied surface irrigation in three sets, water subbed around all plants
6/10 Knocked down furrows and rototilled for weed control; hoe removal of weeds in the rows
6/18 Pulled up furrows and set pipe for irrigation.
7/2-3 Irrigated field as earlier,
8/20-21– Rototilled grapes
9/23-24– Irrigated field as earlier,
10/22- – Rototilled for weed control
Nov 2013 - No activity
Dec 2013 – No activity
1/28-29 2014 – Irrigated field due to lack of normal rainfall.
Feb 2014 – No activity
3/14-15 2014 – Rototilled for weed control and enable rating and pruning
Note: plants were maintained with turgor and soil at field capacity from breaking dormancy to through the winter and bud break in the spring of 2014.

Fungicide and insecticide treatment:

4/19 applied Luna at field rate by fogging, no powdery mildew (PM) symptoms prior to application
5/10 applied second Luna treatment
5/31 applied Inspire at field rate by fogging
6/14 applied Inspire at field rate by fogging
7/3 applied Inspire at field rate by fogging
7/3 there are no foliar powdery mildew symptoms at this point
7/18 Insecticide applied for control of leafhoppers and thrips
Note: no symptoms of powdery mildew or insect damage throughout the year

Publications: 2013 Pierce's Disease Symposium

Presentations: 2013 Pierce's Disease Symposium

Research relevance. The objective is to evaluate transgenic grape and grape rootstocks expressing various genes from different constructs in a field site in Solano County for protection against Xylella *fastidiosa* (Pierce's Disease strain) following mechanical injections of *X. fastidiosa* into the grape canes of both transgenic and co-planted non-transgenic control plants.

Laypersons summary

The purpose of the field planting is to evaluate grape and grape rootstocks expressing several transgenes from several investigators, with differing putative modes of action against *Xylella fastidiosa*, under natural field conditions for efficiency in providing protection against Pierce's Disease. The site in Solano County was selected and approved by APHIS to enable controlled inoculation and close monitoring of the host response in terms of symptoms, bacterial behavior, and plant morphology. Over the course of the multi-year field evaluation, test plants included ungrafted conventional Thompson Seedless and Freedom plants as controls, transgenic plants from investigators Dandekar, Labavitch, Lindow and Gilchrist and later transgenic rootstocks expressing some of the test genes were grafted to untransformed PD susceptible scions to assess potential for disease suppression in an untransformed scion from signals originating in the transformed rootstocks. We propose to continue maintaining and collecting data from this site for the coming 2 years through June 30, 2016. This time period matches the time extension proposed by Dr. Dandekar, who has now assumed responsibility for the APHIS permit. Dr. Gilchrist will continue to manage the field operations at this site. The following budget will cover all the cost of continuation of the experiment and removal of the plants at the end of the experimental period. The APHIS permit specifies that the plants are to be removed, burned on site and the field monitored as fallow for an additional year. There is no cost for monitoring after removal.

Intellectual Property: Evidence for any and all transgenes that show protection against PD will be submitted as a record of invention to the respective Technology Transfer offices at UC Davis and UC Berkeley as first step in protecting patent rights.

Status of funds. Funds are being expended in accordance with the project proposal and budget.

Images below show status of these plantings over the course of the experiment from early summer 2010 through March 2014

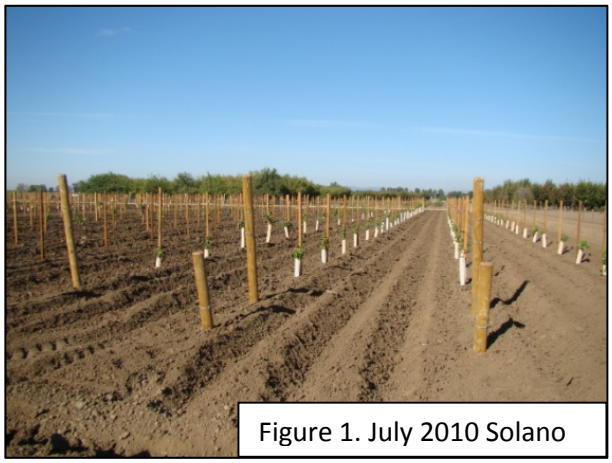

Figure 1. July 2010 Solano

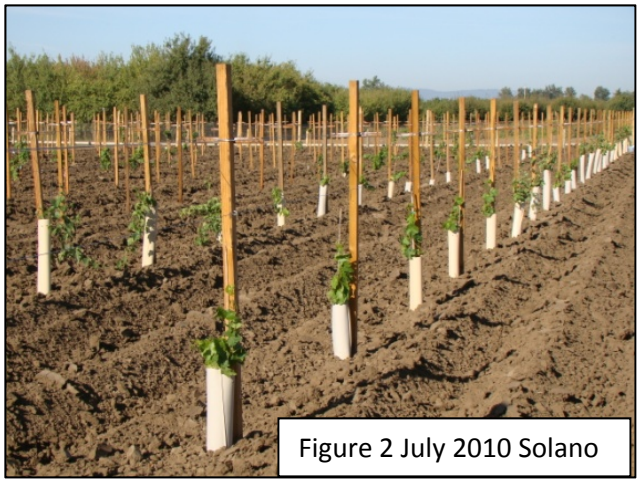

Figure 2 July 2010 Solano

Figure 3 April 2012 Solano

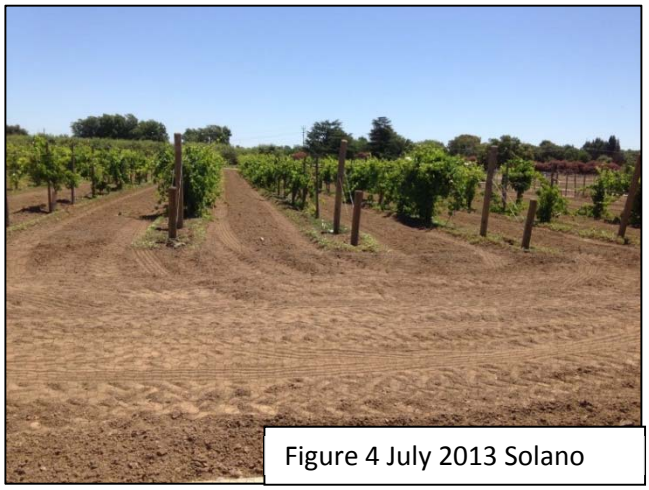

Figure 4 July 2013 Solano

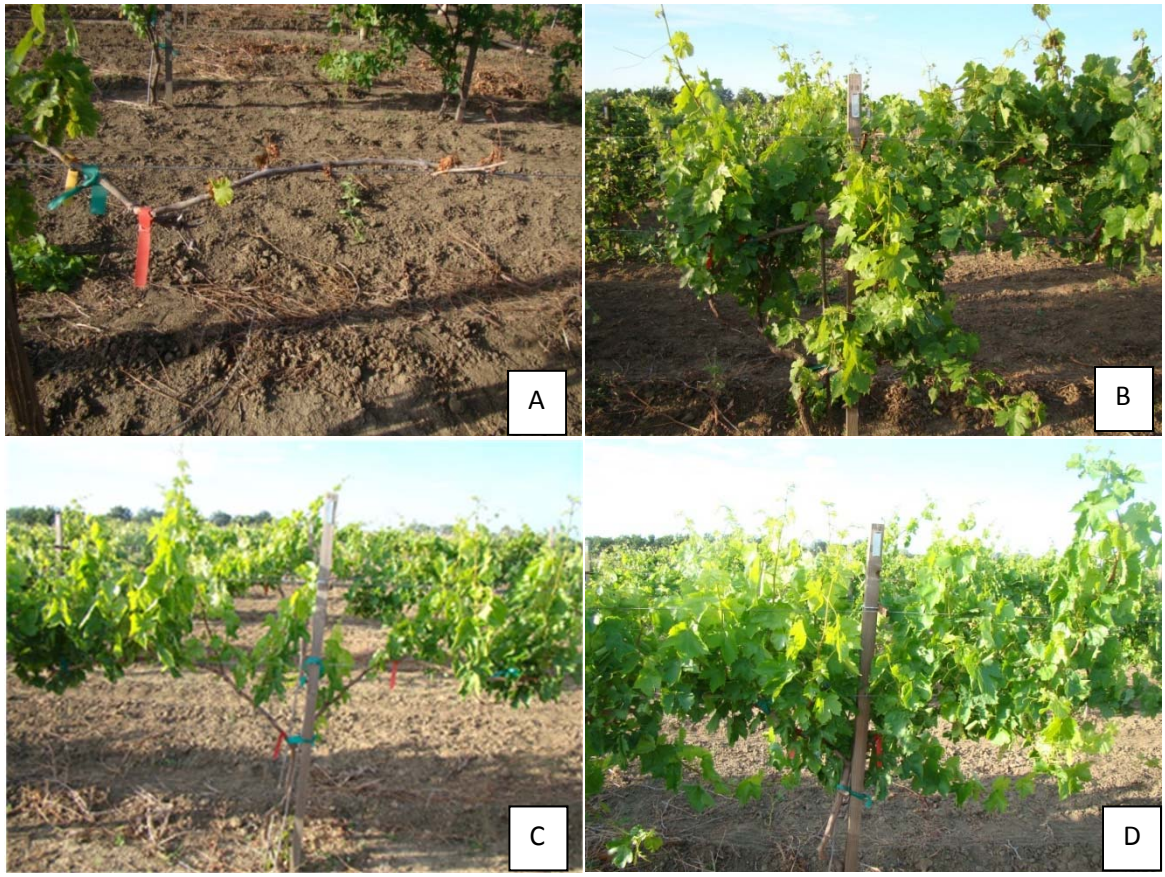

Figure 5. Examples of Solano County field trial images from the Gilchrist plots showing both protection and death of control plants. Colored tags indicate site and date of inoculation: (A) Non-transgenic control plant (TS02A) Inoculated in 2011 and 2012. Image taken April 2013 showing shoots dying shortly after emergence. (B) Inoculated PR1-9 transgenic Thompson Seedless harboring Xf but asymptomatic two years after inoculation. (C) Non- transgenic grafted control plant (TS02A) inoculated July 2012 showing cane death visible on inoculated canes but not uninoculated canes on the same plant. Image illustrates the observation that the bacteria can kill inoculated canes but do not move rapidly to other canes on the same plant. (D) Non-transgenic Thompson Seedless scion grafted to a transgenic PR1-9 rootstock. The scion was inoculated in 2012 and shows no evidence of death in inoculated canes or shoots emerging from the inoculated canes through August 2013.