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.

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Reporting Period: The results reported here are from work conducted January 2014 through July 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 multi-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 were introduced in 2011 and 2012. All plants are localized in an APHIS-approved field area with no risk of pollen or seed dispersal. The area is adjacent to experimental grape plantings that have been infected with Pierce's Disease for the past two decades with no evidence of spread of the bacteria to uninfected susceptible grape plantings within the same experiment. 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 and for the granting of the APHIS permit. The field area chosen has never had grapes planted therein in an attempt to avoid any potential confounding soil borne diseases, including nematodes.

Objectives

A. Land preparation, planting, and management of the experimental resources to accommodate 500 plants. Plants occur 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. Drip irrigation system was installed in 2014 and will be used in all future plantings. 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 works 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 permitting sun exposure to leaves on inoculated canes. Periodic pruning is necessary, given that the transgenic plants are derived from Freedom (common rootstock) and Thompson Seedless both of which exhibit tremendous vegetative growth during the season. In addition, annual pruning will deviate from conventional practice in that multiple cordons will be established with a separate new cordon retained for each successive inoculation. This will enable differential experimental materials in the form of seasonal canes associated each succeeding annual inoculation. The objective is to provide sufficient inoculated and control material for destructive sampling over years to assess both timing of symptom development after successive inoculations and to assess bacterial movement over time.

C. Irrigation and pest management has been provided by Cooperator Tom Kominek as needed. Irrigation from 2010 to current has been by surface flooding. Drip irrigation will be possible on all new plantings beginning in 2014. All management decisions and timing is coordinated with and by PI Gilchrist

D. Plants have been mechanically inoculated with *Xylella fastidiosa* by the Investigators beginning in 2011 and subsequent years. Uniform inoculum will be produced by PI Gilchrist and provided to all investigators. All inoculations occurred simultaneously by all investigators. Hence, inoculum type, concentration and timing will be uniform across all grape plants.

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) with all plants surviving the winter as shown in Figure 2.. The second phase of the planting, including grafted transgenics was completed May, 2011 and June of 2012.

Extensive polish pruning during the season was quickly recognized as necessary to manage the Freedom and Thompson Seedless plants in a fashion to allow ease of mechanical inoculation and recovery of experimental samples (Figure 3).

As of July 21, 2014, all individuals transgenic, exhibited a normal phenotype, true to the untransformed control plants of each parental genotype (Figure 4). Symptoms of Pierce's Disease did not appear until two years after inoculation. Evaluations in the summer of 2014 indicate inoculated controls and some transgenic plants show symptoms of PD. It is clear that this field planting will provide important data on the effectiveness of any of the transgenic strategies employed by the respective researchers.

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. Visual observation and destructive sampling of inoculated canes indicates that mechanical inoculation was successful in infecting inoculated canes (Figure 5). As of July 2014, in several uninoculated canes, adjacent to inoculated canes show foliar symptoms indicting that the bacteria have moved systemically through the plants and, in the case of some non-transformed control plants, the entire plant is now dead.

There are two points to be made regarding the appearance of symptoms. First, plant turgor has been maintained throughout the growing season with timely irrigation and there has not been any evidence of wilt symptoms prior to appearance of classic foliar symptoms (Figure 7) or even death of inoculated control susceptible canes (Figure 6). Symptomatic leaves occur on inoculated canes without the appearance of water stress. This belies the effect of vascular plugging leading to the classic foliar symptoms of sectored death within green areas of leaves. Second, excellent symptoms associated with the presence of the pathogenic bacteria are readily seen in the spring of each year from buds emerging on inoculated canes. Buds break, push tiny leaves, and then die in tissues confirmed in the laboratory to harbor bacteria from inoculations that occurred one to two years prior.

As of July 2014, it is clear that there is a rich source of additional data to be collected from this field experiment. There are now substantial differences between inoculated control plants compared with plants expressing some of the transgenes. There is no evidence of any spread of the bacteria from inoculated to non-inoculated control plants but there is now evidence of systemic spread within some of the plants representing different genetic composition (different transgenes). The positive result of effective mechanical inoculation over time suggests that plants consisting of transgenic root stocks grafted to non-transgenic scions will enable experimental assessment of cross-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 are now approved and funded to continue maintenance and data collection 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.

Details of field operations performed on Solano Pierce's Disease Field experiment from January 2014 to July 2014.

Date: Activity

1/25/14 rototilled and pulled furrows
1/28/14 surface irrigated entire plots….lack of rainfall
2/1-2/28/2014 no further activity
3/14 Tilled for weed control, grapes just beginning to push
3/18-20 Began pruning, carefully leaving all inoculated/tagged branches, PI 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.

4/1-3 field crew hand hoe weeds within rows, PI sampled canes. 30hrs,
4/9 Sprayed Luna for powdery mildew. 8.6 oz ai/ac. 2h/1.5 equipment
4/21-14 field crew hoe weeds and trimmed carefully to expose inoculated branches
4/30 Sprayed Luna
5/1 Rototilling and plot cleanup 8.5h
5/12 Trimming overgrowth and weeding 14 hrs
5/15-17 Rototilled to incorporate trimings and for weed control
5/27 Sprayed Inspire for powdery mildew control. No evidence of PM
5/27 Pulled up furrows in preparation for surface irrigation
5/28 Inoculated all plots with Xylella Temecula strain Gilchrist provided inoculum to all PIs and all plants inoculated same morning.
6/1 Applied surface irrigation in three sets, water subbed around all plants
6/10 Knocked down furrows, additional trimming, rototilled to incorporate, and for weed control; hoe removal of weeds in the rows
6/12 Sprayed Inspire on all plants for powdery mildew control
7/2-3 hand hoe for weeks within rows
7/9 Pull furrows
7/10 sprayed Inspire spraying will continue on approximately 3 week intervals
7/11 Irrigated field as earlier,
7/20-21– Rototilled to incorporate recent trimmings and for weed control

Fungicide and insecticide treatment:

4/9 applied Luna at field rate by fogging, no powdery mildew (PM) symptoms prior to application
4/30 applied second Luna treatment
5/27 applied Inspire at field rate by fogging
6/12 applied Inspire at field rate by fogging
7/10 applied Inspire at field rate by fogging
7/18 Insecticide applied for control of leafhoppers and thrips

Note: no symptoms of powdery mildew or insect damage thus far 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. Dr. Dandekar, has now assumed responsibility for the APHIS permit and Dr. Gilchrist will continue to manage the field operations at this site.

Intellectual Property: Evidence for any and all transgenes that show protection against PD will be submitted by individual PIs 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 July 2014

Figure 1. July 2010 Solano

Figure 2. March 2011 Solano

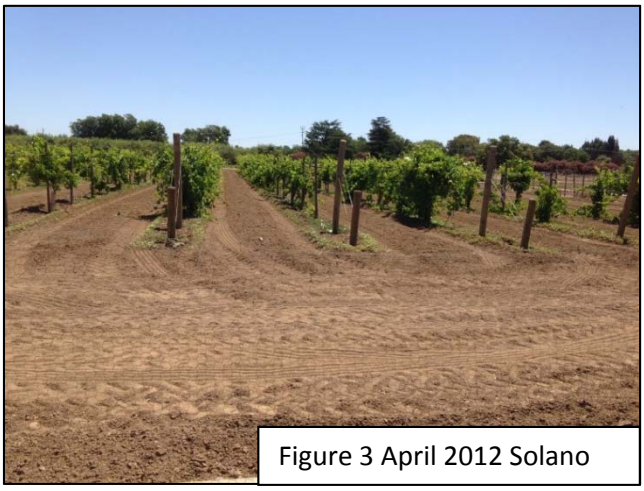

Figure 3 April 2012 Solano

Figure 4 July 2014 Solano

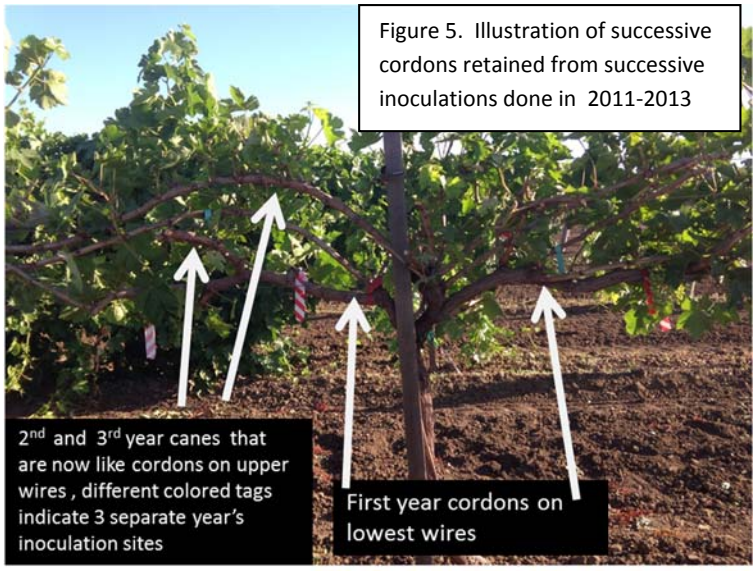

Figure 5. Illustration of successive cordons retained from successive inoculations done in 2011-2013

Figure 6. Illustration of spring PD symptoms where buds or very young shoots die shortly after emergence

Figure 7. Illustration of classical PD foliar symptoms observed July 2014 resulting from mechanical inoculation of Xylella fastidiosa at the Solano site