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 October 2015 to September 30, 2016

INTRODUCTION

This field project began in 2010 to evaluate grapevines expressing potential Pierce's Disease (PD) suppressive transgenes under field conditions. All plants are located in a secured, USDA-APHIS-approved, area in Solano County. The disease was successfully introduced into the cordon trained plants by mechanical injection of *Xylella fastidiosa* into stems over the past five years. The plants were monitored regularly for quantity and movement of the bacteria along with symptoms of PD. Test plants included transgenic plants expressing genes from Dandekar, Powell, Lindow, Gilchrist and Kirkpatrick projects compared with non-transgenic PD-susceptible Thompson Seedless and Freedom rootstock plants as controls. In addition, transgenic rootstocks expressing some of the test genes grafted to untransformed PD susceptible scions were introduced in 2011 and 2012. The results to date indicate that the mechanical inoculations introduced the bacteria into the plants with subsequent appearance of classic foliar symptoms and cane death within 24 months in susceptible controls. There is no evidence of spread of the bacteria to uninoculated and uninfected susceptible grape plants adjacent to infected plants, confirming tight experimental control on the pathogen and symptoms. Each of the transgenes tested suppress the symptoms of PD inoculated vines to varying degrees, including protection of untransformed scions on the grafted plants. This field research is moving forward with the generation of new transgenic rootstocks expressing pairs of the disease suppressive genes in a gene stacking approach with the genes paired together by differential molecular function. The new rootstocks with two transgenes each will be evaluated first in the

laboratory and then the greenhouse before moving to the field. The highest expressing rootstocks will be grafted to susceptible non-transgenic scions to assess potential cross graft protection against PD. Funding has been provided by CDFA/PD/GWSS program with this project approved for a no-cost extension through June 30, 2017.

OBJECTIVES

There are three principal objectives for the duration of this project under 12-0444-SA and which is being continued under renewed funding by the PD Project.

1. Complete the current field evaluation of transgenic grape and grape rootstocks expressing Pierce's Disease suppressive DNA constructs in the APHIS regulated field site in Solano County through the spring of 2016.
2. Remove the current planting per the APHIS agreement by dismantling trellising, uprooting the plants and burning all grape plant material on site, weather permitting, in the fall of 2016 and winter of 2017, followed by cultivation and fumigation to ensure no living grape vegetative material remains.
3. Establish a new planting area within the current APHIS approved site (figure 6) to contain a new set of lines bearing paired, PD suppressive, DNA constructs, referred to as stacked genes. The stacked genes will be transferred to two adapted grape rootstocks (1103 and 101-14). These rootstocks will be grafted to a PD susceptible Chardonnay scion prior to field planting. The goal is to assess the potential of cross graft protection against PD of a non-transgenic scion. Planting to begin in 2016 and completed by 2018.

The field experiment funded under 12-0444-SA will be terminated under objectives 1 and 2 of this proposal according to the regulations specified in the APHIS permit. This will be followed by establishment of second phase approved by the Product Development Committee to develop transgenic rootstocks incorporating stacked genes (dual constructs) to be grafted to non-transformed PD-susceptible Chardonnay scions to test for potential cross-graft protection against PD (Objective 3). The development of the stacked gene rootstock transgenics is in progress, including molecular analysis of several lines released by the UC Davis Transformation Facility. The second phase also involves limited planting and inoculation of additional single DNA constructs not previously tested. The second phase planting and inoculation will begin in 2016 to be concluded in 2018. All field activities described in the below will be coordinated by Dr. Gilchrist through field superintendent Bryan Pellissier.

DESCRIPTION OF ACTIVITIES TO ACCOMPLISH OBJECTIVES

1. Destruction of existing planting and fumigation of the area to permit future use will first involve removal of all stakes and trellis, followed by cutting and stacking the above ground portions of the plants. Mechanical undercutting of the base of the plants and roots will complete the plant removal. The stacked plants will be burned on the site inside the APHIS permitted area. Following burning, the ashes will be scattered and the entire area rototilled prior to fumigation to complete the APHIS requirements for removal and destruction of all transgenic material.
2. Establishment and management of new planting (Figure 6): Mark Greenspan (PD Board viticulture consultant) will work with PI Gilchrist to develop the following approach for trellising and plant management to reflect commercial standards and to enable the experimental inoculations, pathogen and disease assessments, as well as grape yield. Land preparation and planting of the experimental area will be sufficient to accommodate and manage 900 new plants. Row spacing will be 9 feet between rows with 6 feet between plants. This spacing permits 32 rows of 28 plants each (up to 896 plants total) and includes a 50 foot open space around the planted area as required by the APHIS permit. The planting pattern will permit a 2 bud pruned bilateral cordon system of sufficient lengths for inoculation, real time sampling of inoculated tissue and determination of the fruit yield by the untransformed Chardonnay

scions. Total fenced area occupied by plants and buffer zones as required by the APHIS permit will be ~3.4 acres. All plants will be maintained under a drip irrigation system that was installed in 2014.

RESARCH RELEVANCE

This field project began in 2010 to evaluate grapevines expressing potential Pierce's Disease (PD) suppressive transgenes under field conditions. All plants are located in a secured, USDA-APHIS-approved, area in Solano County. The disease was successfully introduced into the cordon trained plants by mechanical injection of *Xylella fastidiosa* into stems over the past four years. The plants were monitored regularly for quantity and movement of the bacteria along with symptoms of PD. Test plants included transgenic plants expressing genes from Dandekar, Powell, Lindow, Gilchrist and Kirkpatrick projects. The transgenic plants were compared with non-transgenic PD-susceptible Thompson Seedless and Freedom rootstock plants as controls. In addition, transgenic rootstocks expressing some of the test genes grafted to untransformed PD susceptible scions were introduced in 2011 and 2012. The results to date (Figures 1-5) indicate that the mechanical inoculations introduced the bacteria into the plants with subsequent appearance of classic foliar symptoms and cane death within 24 months in susceptible controls. There is no evidence of spread of the bacteria to uninoculated and uninfected susceptible grape plants adjacent to infected plants, confirming tight experimental control on the pathogen and symptoms. Each of the transgenes tested suppress the symptoms of PD inoculated vines to varying degrees, including protection of untransformed scions grafted to a transformed rootstock. The field evaluation is being continued in the expanded area shown in Figure 6 under the existing APHIS permit with transgenic rootstocks expressing two "stacked" genes grafted to a non-transgenic PD-susceptible scion to assess the potential for cross-graft protection of the scion.

LAYPERSONS SUMMARY

This field project began in 2010 to evaluate grapevines expressing potential Pierce's Disease (PD) suppressive transgenes under field conditions. This field experiment will continue evaluation of resistance to Pierce's Disease (PD) in transgenic grape and grape rootstocks by expressing dual combinations of five unique transgenes under field conditions. The evaluations continue in an USDA-APHIS-regulated Solano County site where the plants are mechanically injected with *X. fastidiosa*. Pierce's Disease symptoms including classical foliar symptoms and cane death occur within 24 months. The current field tests have shown positive protection against PD by five (5) different DNA constructs. A new planting is in progress that will consist of untransformed PD susceptible scions grafted to transgenic rootstocks (1103 and 110-14) expressing the paired constructs of the five genes to assess cross-graft protection of a non-transformed scion that is otherwise highly susceptible to Pierce's Disease..

Funding Agencies and Status of Funds: Funding for this project is provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board and the Regents of the University of California. This project, 12-0444-SA, has been approved for a no cost extension to June 30, 2017 but the research effort will continue under the project of the same title for which funds were released in November 2016.

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.

CONCLUSIONS

The current planting of transgenic grapes will be terminated and the plants removed in the fall of 2016. Remove the current planting per the APHIS agreement by dismantling trellising, uprooting the plants and burning all grape plant material on site in the fall of 2016, followed by cultivation and fumigation to ensure no living grape vegetative material remains.

The field research using PD suppressive transgenes is moving forward with the generation of new transgenic rootstocks expressing pairs of the disease suppressive genes in a gene stacking approach with the

genes paired together by differential molecular function. The new rootstocks with two transgenes each will be evaluated first in the laboratory and then the greenhouse before moving to the field. The highest expressing rootstocks will be grafted to susceptible non-transgenic scions to assess potential cross graft protection against PD. The field area has been permitted by the USDA-APHIS for this experiment. The protocol for planting and management of the vines is in place and is coordinated with Mark Greenspan (PD board Consultant). Four years beginning with initial planting in the spring of 2016 and followed by addition plantings as experimental plants become available in the second and third years. Inoculation and evaluation will begin when the plants have been in the ground for one year and will continue annually until the field planting is terminated. Funding for completion of the fourth and any following years will be proposed in the 2018-2019 funding cycle and will depend on the results of the field evaluation up to that point.

Publications: 2016 Pierce's Disease Symposium and 2015 Summary of Accomplishments posted on the CDFA Pierce's Disease website in lieu of a symposium-based report.

Images below show status of these plantings over the course of the experiment from early summer 2010 through August 2016

Figure 1 July 2010 Solano planting

Figure 2 July 2012 Solano planting

Figure 3. Symptoms of Pierce's Disease recorded July 21, 2015 on branches inoculated May 27, 2014.

Figure 4. Thompson Seedless untranformd scion grafted to a 456-8 transformed Thompson seedless rootstock in 2012, 2013, and 2014

Figure 5 Thompson Seedless untransformd scion grafted to a P14 transformed Freedom rootstock inoculated in 2012, 2013, and 2014

Figure 6. Solano planting area. Future area (green) available to plant the next generation of transgenic plants expressing the dual constructs or new single genes: This area is 300 X 470 ft for planting, which equals 1.8 acres accommodating up to 38 new rows (excluding the 50 ft buffer areas surrounding the plots. The new area will accommodates ~1000 new plants in 2016-18. Current area (rows) now planted to grapes: 300 X 370 ft equaling 1.6 acres including the 50 ft buffer areas surrounding the plots. There are currently 625 plants that have been evaluated since 2010. These plants will be removed in 2016. After the plants are removed and destroyed by burning, the area will be fumigated and available for additional new plantings or a rootstock nursery by 2018