Final report for CDFA Agreement Number 12-0444-SA

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

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Important information on continuation of this project: Project 12-0444-SA was given a no cost extension from 07/01/2016 to 06/30/2017.The results reported here cover the entire period of this grant from 02/01/2013 to 06/30/2017. It is important to note that, even though funding for this project ended 06/30/2017, the objectives and ongoing data collection of this field research are being continued with minor modifications to the title and without interruption under a new grant, CDFA-16-0558-SA.

INTRODUCTION

This field project began in 2010 to evaluate grapevines expressing potential Pierce's Disease (PD) suppressive transgenes under field conditions and is continuing with subsequent renewals. The field is 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 (Xf)* into stems beginning in 2011. 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 a small contingent of plants from the late Bruce Kirkpatrick. The response of transgenic plants to *Xf* 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 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 was 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 planting was terminated with the removal and final burning of the plants on June 7, 2017. However, this field research is being continued 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. Following evaluation of new rootstocks with two transgenes each, they will be evaluated first in the laboratory and greenhouse in 2017 before moving to the field in 2018. The highest expressing rootstocks will be grafted to susceptible non-transgenic Chardonnay scions to assess potential cross graft protection against PD. Funding for the continuation has been provided by CDFA/PD/GWSS program through a new grant, CDFA-16-0558-SA.

OBJECTIVES

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 by the spring of 2017. Following complete destruction, the filed will be fumigation to ensure no living grape vegetative material remains (Figure 1).
3. Establish a new planting area within the current APHIS approved site (figure 3) 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 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 derived from a non-transgenic scion. Planting to begin in 2017 and completed by 2018.

In conjunction with the investigators, the Product Development Committee of the Pierce's Disease Control Board in October 2015, approved the decision to terminate the field evaluation of current transgenics as originally planned and move to the second phase of transgenic PD resistance evaluation. Field data over the course of this experiment has been collected by all investigators and can be found in their individual reports from in the 2012- 2016 Pierce's Disease Symposium reports.

The ongoing field experiment has been terminated under objectives 1 and 2 of this proposal according to the regulations specified in the APHIS permit (Figure 1). 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 2017 to be concluded in 2018. All field activities described in the section on Methodology to Accomplish Objectives will be coordinated by Dr. Gilchrist through field superintendent Bryan Pellissier.

DESCRIPTION OF ACTIVITIES TO ACCOMPLISH OBJECTIVES.

Destruction of existing planting was begun in the fall of 2016 (Figure 1). All posts and wires were removed in November but early rains prevented the removal of the plants. Mechanical undercutting of the base of the plants and roots followed by moving the plant material to piles. Final burning occurred on June 6, 2017 and the ashes scattered prior to disking, leveling and fumigation to complete the APHIS requirements for removal and destruction of all transgenic material.

Establishment and management of new planting: will be guided by Josh Puckett and Deborah Golino

Figure 1. Ready for destruction. Plants at Solano field site November 15, 2016 before removal of posts and wires.

Figure 2. Final destruction of the plants at Solano field site by burning on June 7, 2017 following removal of poles and wires, undercutting and piling of plants

(FPMS) working with PI Gilchrist to develop the strategies for producing clones for grafting non-transgenic scions, grafting, field planting, 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 (Figure 2)

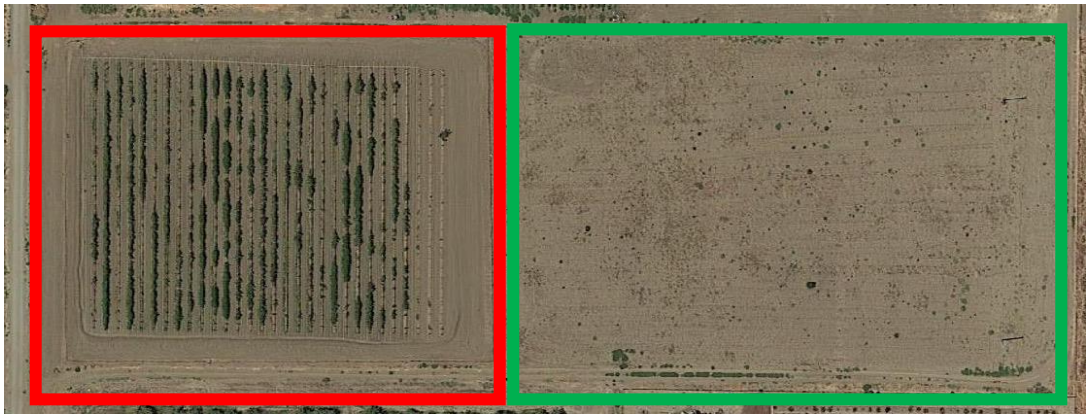

Figure 2. Solano planting area. Future area (green box) available to plant the next generation of transgenic plants expressing the dual constructs or new single genes: This area is 300 X 470 ft for planting, which equals 1.8 acres accommodating up to 38 new rows (excluding the 50 ft buffer areas surrounding the plots. The new area will accommodates ~900 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.

All future plants will be maintained under a drip irrigation system. The initial planting and installation of the drip irrigation is shown in Figure 3.

Research timetable for the new planting of dual constructs and untested single constructs. Four years beginning with initial planting in the spring of 2018 (Figure 3) to be 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. The field area has been designated legally available for planting the specified transgenic grapes by USDA-APHIS under permit number 7CFRE340 that is held by Professor Abhaya Dandekar. The protocols for managing the existing and the new plantings with the dual constructs have used successfully over the past 5 years (Gilchrist 2015a). These protocols include the plant management, inoculation with *Xylella fastidiosa,* development of classical symptoms of Pierce's Disease exhibiting the range from foliar symptoms to plant death and the assessment of protection by a set of transgenes selected by molecular techniques to suppress the symptoms of Pierce's Disease and/or reduce the ability of the pathogenic bacteria to colonize and move within the xylem of the grape plant.

CONCLUSIONS

The current planting of transgenic grapes was fully terminated in the spring of 2017 per the APHIS agreement by dismantling trellising, uprooting the plants and burning all grape plant material on site. The complete removal of the plants was followed by cultivation and the area will be fumigated when conditions permit 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 ongoing research is funded under a new grant, CDFA-16-0558-SA. The protocol for planting and management of the vines is in place and will be coordinated by Josh Puckett and Debora Golino. Beginning with initial planting in 2017 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 progress of the field evaluation up to that point.

LAYPERSON 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 evaluation 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.

STATUS OF FUNDS

Funds are being expended consistent with timeline.

SUMMARY AND STATUS OF INTELLECUAL PROPERTY

No intellectual property is has been filed at this point but will be as results dictate

LITERATURE CITED

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