Renewal Progress report for CDFA Agreement Number 16-0558

Field Evaluation of Cross-Graft Protection Effective Against Pierce's Disease by Dual and Single DNA Constructs

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Reporting Period: The results reported here are from September 30, 2016 to April 1, 2017

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.

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 in the fall of 2016, followed by cultivation and 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 of a non-transgenic scion. Planting to begin in 2016 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 field experiment will be 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

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

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 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. The plant removal, burning of the plants and fumigation of the area to permit future use will be accomplished as soon as the field dries. 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.

Establishment and management of new planting: 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 (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 plants will be maintained under a drip irrigation system that was installed in 2014. The initial planting and installation of the drip irrigation is shown in Figure 3.

Figure 3. Newly planted grapes. The new plantings will be watered by drip irrigation.

The following protocols will be followed as the planting proceeds:

a. Experimental design will be a complete randomized block with eight (8) plants per each of five (5) entries (replications), including all controls. Each plant will be trained as a single trunk up the wood stake as with the existing planting. When the shoot tip reaches about 12" past the cordon wire it will be topped to just above a node that is about 2-3 inches below the wire. Then, the laterals that push will be used to establish the bi lateral cordons. Following Marks' advice, the best practice is to let them grow vertically, or close to vertical, rather than tying them while green, which reduces their elongation and tends to force more lateral growth. Metal 9' highway stakes, inserted 3' into the ground every 18' will support the wires, including catch wires. A single 11 gauge wire will be used for the cordons and 13 gauge for the catch wires. Two pairs of moveable catch wires will be installed to tuck and position the shoots vertically for optimizing bacterial inoculation, bacterial analysis, and fruit production. The catch wires will be installed initially or after the first year of growth using 13 gauge wire to support the drip irrigation wire, about 18" off the ground.
b. After the first year, the canes will be tied down during the dormant season and trimmed to the appropriate length or shorter if the cane girth is not over 3/8" in diameter. The shoots that push will be suckered to remove double shoots and to achieve a shoot (and hence spur position) spacing of about 4-5 inches between them.
c. Grape fruit yield will be measured after second or third year depending on the fruit set.

d. Evaluation of the experimental plants for plant morphology, symptoms of Pierce's Disease infection, and the presence of the bacteria will follow past protocol. Each parameter will be determined overtime by visual monitoring of symptom development and detection of the amount and movement of the bacteria in plant tissues (mainly leaves and stems) by quantitative PCR (qPCR) assays. The analysis will be done in the Gilchrist lab by the same methods and laboratory personnel as has been done with the current planting. A comparative quantitative determination by qPCR of the presence of Xylella in non-transgenic scions and grape rootstocks will be compared with conventional grape and grape rootstocks.
e. Both symptom expression and behavior of the inoculated bacteria will provide an indication on the level of resistance to Pierce's Disease infection and the effect of the transgenes on the amount and movement of the bacteria in the non-transgenic scion area.
f. 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, which is to avoid any potential confounding by soil borne diseases, including nematodes.
g. Irrigation and pest management, primarily powdery mildew, weeds and insects, will be coordinated by PI Gilchrist and conducted by Bryan Pellissier the Field Superintendent employed by the Department of Plant Pathology. The field crew work closely with PI Gilchrist to determine timing and need of each of the management practices, including pruning and thinning of vegetative overgrowth as necessary.
h. Regular tilling and hand weeding will maintain a weed-free planting area. Plants were pruned carefully in March leaving all inoculated/tagged branches and numerous additional branches for inoculation and sampling purposes in the coming year. All pruning material was left between the rows to dry, then flail chopped and later rototilled to incorporate the residue per requirements of the APHIS permit.
i. Application of the fungicides Luna Experience and Inspire will be alternated at periodic intervals to maintain the plants free of powdery mildew. Leafhoppers and mites will be treated with insecticides when needed. Neither powdery mildew nor insect pressure was has been observed with these ongoing practices throughout the past five growing seasons.

Research timetable for the new planting of dual constructs and untested single constructs. Four years beginning with initial planting in the spring of 2016 (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 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.

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 filled at this point but will be as results dictate

LITERATURE CITED

Dandekar, Abhaya M. 2015 Chimeric Antimicrobial Protein and Polygalacturonase-Inhibiting Protein Transgenic Grapevine Field Trial. Proceedings of the 2015 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Gilchrist, D.G., and J.E. Lincoln. 2014. Field evaluation of grape plants expressing PR1 and UT456 transgenic DNA sequences for protection against Pierce's disease. *Proceedings of the 2014 Pierce's Disease Research Symposium*. California Department of Food and Agriculture, Sacramento, CA.

Gilchrist, David G. and James E. Lincoln. 2015a Field Evaluation of Grape Plants Expressing Potential Protective DNA Sequences Effective against Pierce's Disease. Proceedings of the Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Gilchrist, David G. and James E. Lincoln. 2015b Field Evaluation of Grape Plants Expressing PR1 and UT456 Transgenic DNA Sequences for Protection against Pierce's Disease Proceedings of the Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Gilchrist, David G., James E. Lincoln, Abhaya M. Dandekar, and Steven Lindow. 2015c Transgenic Rootstock-Mediated Protection of Grapevine Scions by Single and Stacked DNA Constructs Proceedings of

the Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Lindow, Steven. 2015 Continued Field Evaluation of Diffusible Signal Factor Producing Grape for Control of Pierce's Disease Proceedings of the Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

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