

Interim 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 February1, 2013 to July 15, 2013

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 and 2011 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 planned for this project.

OBJECTIVES

- A. Land preparation, planting, and management of the experimental resources: . Land to accommodate 500 plants from the investigators was designated for this project. 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, which is used for other experimental planting not related to this

project. Irrigation and pest management, primarily powdery mildew and insects, is conducted by the Cooperator Tom Kominek, Field Superintendent for the Department of Plant Pathology who has 30 years' experience working with grapes under experimental conditions for USDA and UC Davis scientists.

- B. Principal Investigators with assistance from contract field crews will be 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
- C. Irrigation and pest management will be provided by Cooperator Tom Kominek as needed.
- D. Plants will be mechanically inoculated with *Xylella fastidiosa* by the Investigators in 2011 and subsequent years.

RESULTS AND DISCUSSION

All of the above objectives set out for the establishment of this field planting were completed. 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. The second phase of the planting, including grafted transgenics was completed May 17, 2011.

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.

As of July 21, 2011, the initial 2010 planting and the second 2011 planting individuals are healthy, growing normally and all plants have 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.

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 are still alive. The observation indicates that mechanical inoculation was successful but further suggests that the bacteria have not yet moved systemically through the plants. Plant turgor has been maintained throughout the growing season.

Solano Pierce's Disease Field Work 2013

All field activities are conducted or coordinated by field superintendent Tom Kominek.

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

Note: plants were maintained with turgor and soil at field capacity from breaking dormancy to present

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

Publications: None

Presentations. None

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 stems of transgenic and non-transgenic control plants.

LAYPERSON SUMMARY

The purpose of the field planting is to evaluate transgenic grape and grape rootstocks under natural field conditions for efficiency in providing protection against Pierce's Disease. The site in Solano County will enable controlled inoculation and close monitoring of the host response in terms of symptoms, bacterial behavior, and plant morphology. Over the course of the 3 year field evaluation, test plants in the first planting will include ungrafted conventional Thompson Seedless and Freedom plants as controls, transgenic plants from Dandekar, Labavitch, Lindow and Gilchrist projects and, as plant material from the first planting becomes available, transgenic rootstocks expressing some of the test genes will be grafted to untransformed

PD susceptible scions to assess potential for disease suppression in an untransformed scion from signals in the transformed rootstocks.

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

Intellectual property. None envisioned at this point. All IP is being handled from the previous greenhouse studies under tightly controlled conditions with mechanical inoculation.

Image on the left below show status on 2/27/2013, while the image on the right shows both asymptomatic inoculated transgenic and symptomatic inoculated non transgenic control plants at the Solano County site on June 17, 2013

