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 October 2014 to March 2015

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). The experimental protocol includes mechanical injections of *X. fastidiosa* into the plant stems annually over a 5 year period. 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, Powell, 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 located 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, which is to avoid any potential confounding by soil borne diseases, including nematodes. There is no evidence of spread of the bacteria to uninfected susceptible grape plantings

Methods and 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. Furrow irrigation will be continued on the existing plots, although a drip irrigation system was installed in 2014 and will be used in all future plantings. Irrigation and pest management, primarily powdery mildew, weeds and insects, is coordinated by PI Gilchrist and conducted by Tom Kominek the Field Superintendent employed by the Department of Plant Pathology. Mr. Kominek recently retired after 30 years' service and has been replaced by Mike Eldridge who has 20 years' experience working with grapes and other perennial crops. The field crew work 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 trimming is necessary, given that the transgenic plants are derived from Freedom (a common rootstock) and Thompson Seedless both of which exhibit tremendous vegetative growth during the season. In addition, annual pruning deviates from conventional practice in that multiple cordons have been established with a separate new cordon retained from each successive inoculation. This enables differential experimental materials for evaluation and sampling 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 presence and movement over time.

C. Plants have been mechanically inoculated annually with *Xylella fastidiosa* beginning in 2011 and will be mechanically inoculated again in 2015

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 in 2010. 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 trimming 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 March 2014, 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 been no evidence of wilt or epinasty symptoms prior to appearance of classic foliar symptoms or even death of inoculated control susceptible canes. Symptomatic leaves occur on inoculated canes without the appearance of water stress.. This belies the long held anecdotal effect of vascular plugging leading to the classic foliar symptoms of sectored death within green areas of leaves. Second, in 2014, definitive 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 September 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 from the 2014 Pierce's Disease Symposium.

As of March 15th, 2015, all plants have been pruned to remove excess growth from the past year but to retain all inoculated wood and spurs on the wood. Spurs on old inoculated cordons were pruned to 2-3 buds while the 2014-inoculated branches were trimmed to retain up to 10 buds for data collection to include live/dead bud counting and destructive sampling for bacterial counts.

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.

Solano County Pierce's Disease Field Work 2014 to be continued in 2015: All field activities are conducted or coordinated by field superintendent Mike Eldridge and PI Gilchrist. Regular tilling and hand weeding 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. Frequent trimming of the plants is done to ensure that leaves on inoculated canes were exposed to sunlight and shading of the associated leaves was avoided. Surface irrigation was applied as needed to maintain the soil at field capacity and turgor in the plants. Application of the

fungicides Luna Experience and Inspire were alternated at periodic intervals to maintain the plants free of powdery mildew. Leafhoppers and mites were treated with insecticides when needed. Neither powdery mildew nor insect pressure was noted throughout the growing season. The same maintenance program is scheduled for 2015.

Conclusions

The results to date of this field experiment indicate that the mechanical inoculations successfully introduced the bacteria into the plants with subsequent appearance of foliar symptoms and cane death. There are transgenes from each of the investigators that appear to be suppressing the symptoms of PD inoculated vines.

Images below illustrate the status the field experiment from planting in 2010 to the summer of 2013. The caption to each figure indicates the date the image was obtained and together they represent the both asymptomatic inoculated transgenic and symptomatic inoculated non transgenic control plants at the Solano County site.

Publications: 2014 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. We will continue maintaining and collecting data 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. The APHIS permit specifies that the plants are to be removed, burned on site and the field monitored as fallow for an additional year. This latter step may be modified if there is additional planting at this site. Additional space has been set aside to enable doubling of the current work area with a modification of the current permit.

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.

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

Images below show status of these plantings over the course of the experiment from early summer 2010 through March 2015

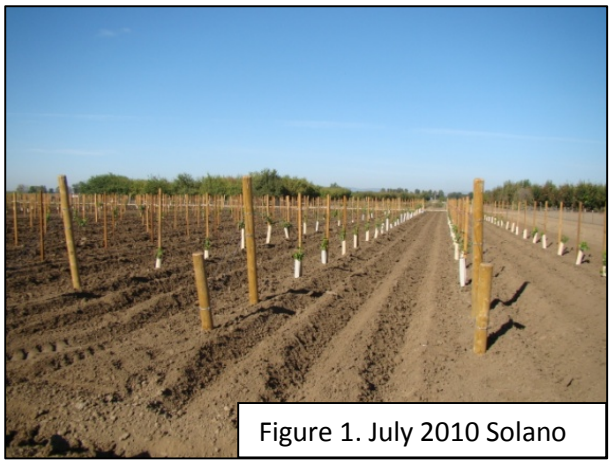

Figure 1. July 2010 Solano

Figure 2 July 2010 Solano

Figure 3 April 2012 Solano

Figure 4 July 2013 Solano

Figure 5. Solano County field trial images from the Gilchrist plots taken on March 15, 2015 showing plants have been pruned to remove excess growth from the past year (A) but to retain all inoculated wood and spurs on the wood. Spurs on old inoculated cordons were pruned to 2-3 buds while the 2014 inoculated branches were trimmed to retain up to 10 buds for data collection to include live/dead bud counting and destructive sampling for bacterial counts (B).

March 16, 2015 Solano. Genotype is Freedom with no bud emergence at this date.

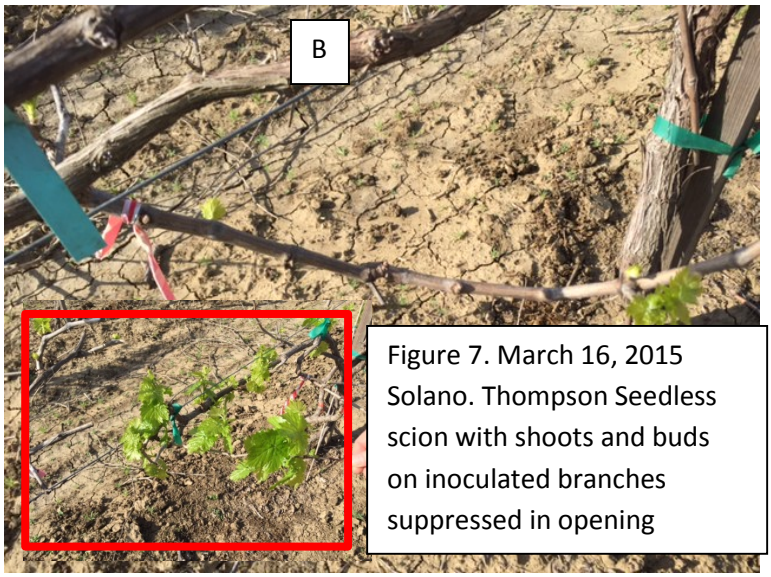

Figure 7. March 16, 2015 Solano. Thompson Seedless scion with shoots and buds on inoculated branches suppressed in opening

Figure 6. Panel A shows a control Freedom rootstock compared with panel B showing an example of a transgenic Thompson Seedless plant. The Freedom plants are 1-2 weeks later in emergence than the Thompson plants. The inset images in panel B illustrates leaf emergence on a non-inoculated non-transgenic Thompson Seedless plant compared with an 2014 inoculated shoot illustrating a consistent observation that buds on inoculated shoots are delayed in emergence at this point in time. Detailed rating and destructive sampling will be completed within the next two weeks to assess live/dead buds on these shoots across all genotypes and bacterial presence.