Interim Progress Report March for CDFA Agreement number 12-0443-SA

Field evaluation of grape plants expressing PR1 and UT456 transgenic DNA sequences for protection against Pierce's Disease.

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Time period covered by the report October 2015 to March 1, 2016

Project History: Initiated in 2010 with continuation to June 30, 2016.

INTRODUCTION

Field experiments in Solano County will evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs, PR1 and UT456, in several different transgenic lines of each construct for resistance to the Pierce's Disease strain of *Xylella fastidiosa* (*Xf*). The background research on selected transgenic lines leading to these field trials is from four controlled inoculation experiments in a greenhouse over a two year period, involving more than 300 transgenic plants of five lines derived from independent transformation events bearing PR1and UT456. Each of these transgenes in several lines suppressed PD symptoms and reduced bacterial titer compared with untransformed controls of the same genotype. A positive correlation between the PR1 and UT456 message level, suppression of bacterial titer and absence of PD symptoms was established using qPCR to measure both the message and the bacteria titer.

Mechanical inoculation of *Xf* is employed at the Solano site to establish controlled infection in the experimental plants. The first phase of the field studies started in 2010 to evaluate clonal copies of the fully transformed own-rooted plants that exhibited suppressed PD symptoms and low bacterial titers in greenhouse assays (1). The second phase began in 2011 with planting the untransformed Thompson Seedless scions grafted onto PR1 and UT456 primary transformants as rootstocks, including Freedom and Thompson Seedless. Data collected in 2012-15 indicate that the bacteria are present in the mechanically inoculated canes on plants at the Solano site (2, 3). Results indicate that both PR1 and UT456 transgenes provide protection against PD, while the level of protection varies between individual transgenic lines. Field symptom data was collected in the fall of 2014-15. As of March 15, 2015, the plants were pruned to remove excess dormant branches while leaving portions of previously inoculated cordon-like branches dating back to 2015 (Figure 1). In the latter case, up to 10 buds were preserved on each inoculated cane as a data rich resources for scoring dead vs live buds on control and transgenic plants as new shoots begin to emerge in the spring of 2015 (Figures 2, 3 and 4). The 2014 inoculated canes were destructively sampled to determine the presence and concentration of *Xf* in the tissues after scoring the new buds (Figure 5).

OBJECTIVES

The overall objective is to continue to evaluate several lines of transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 for resistance to the Pierce's Disease strain of *Xf* at a site in Solano County.

- Visual assessment of plant phenotype comparing the transgenic plants with the untransformed

wild plants which are Thompson Seedless and Freedom

- Controlled mechanical inoculation of *Xf* is used at the Solano County site to initiate infection and develop PD symptoms in the absence of the glassy-winged sharpshooter and no plant to plant spread by other means.
- A first planting of fully transformed plants was established in 2010 and a second set of plants consisting of rootstocks transformed with PR1 and UT456 genes grafted to untransformed PD susceptible Thompson Seedless scions. The grafted plants are designed to assess the potential for trans-graft protection against PD.
- Disease rating: The data collected includes date and timing of symptom expression, including live/dead bud counts in the spring as the buds are emerging.
- Quantitative analysis of Xf in the inoculated canes of the transgenic plants and the untransformed control plants by qPCR.

DESCRIPTION OF ACTIVITIES TO ACCOMPLISH OBJECTIVES.

Plant phenotypes: There were no distinguishable morphological differences in the control plants compared with any of the transgenic lines using criteria of descriptors described by the International Organization of Vine and Wine. All plants have a normal phenotype, true to the untransformed control plants of each parental genotype and all produced abundant fruit. The Thompson Seedless transgenic plants are fully fruited with no visually distinguishable differences in fruit set, fruit size or maturity from the untransformed control plants. The field map in Figure 6 shows the genotypes and colored bars indicating the various inoculation dates and bacterial populations introduced at each inoculation date. By late June of 2014 all the inoculated untransformed control plants showed foliar symptoms of PD, along with some of the experimental plants. Uninoculated control plants appear healthy in all cases indicating no spread of disease from inoculated to uninoculated untransformed susceptible plants.

Xf titers by qPCR:

Inoculated plants were confirmed to have been successfully infected in the 2011, 2012, 2013 and 2014 inoculations by sampling individual inoculated canes followed by qPCR analysis for relative bacterial populations. Bacterial numbers from inoculated plants not showing symptoms varied from 500-1500 cells per 1 cm of inoculated stem tissue. The inoculations on non-transgenic plants showing symptoms ranged from 10^4-10^6 cells per 1 cm of inoculated stem tissue. Example data from the untransformed Thompson Seedless scions grafted to the transformed rootstocks bearing either PR1 or UT 456 transgenic DNA sequences is presented in Figure 5.

Disease ratings 2014 and 2015:

By late June of 2015 all the inoculated untransformed control plants showed foliar symptoms of PD, along with some of the experimental plants (Figures 6 and 7). Uninoculated control plants appear healthy in all cases. There is no evidence of plant to plant spread and only limited movement of bacteria from an inoculated cordon to uninoculated adjacent cordons or canes. The young canes of untransformed scions grafted to transgenic rootstocks, inoculated in May 2014, began to show PD symptoms within 90 days. Eight leaves from the point of inoculation were rated for foliar symptoms at 120 days revealed significant differences PD symptoms between control and transgenic rootstocks.

As of March 15 2015, the plants were pruned to remove excess dormant branches (Figure 1) while leaving portions of previously inoculated cordon-like branches dating back to 2011and last years inoculated canes. In the latter case, up to 10 buds were preserved on each inoculated cane as data rich resources for scoring dead vs live buds on control and transgenic plants as new shoots begin to emerge in the spring of 2015 (Figures 2, 3 and 4). The 2014 inoculated canes were destructively sampled to determine the presence and concentration of *Xf* in the tissues after scoring the new buds on the grafted plants (Figure 5).

Results from the analysis of bacteria presence, movement, and preseince of PD symptoms indicate that both PR1 and UT 456 protective sequences function to suppress PD symptoms in the fully transformed Thompson Seedless and Freedom plants and the protective effect may move across a graft union to protect an untransformed and susceptible wild type scion. Both the PR1 and UT456 expressing plants show suppression of symptoms and reduced bacterial counts. Individual plants within several of the UT456 and PR1 lines have remained asymptomatic while some lines are less suppressive all lines are rated more suppressive of PD than the controls. This project has identified a basis for PD symptoms and a genetic mechanism to suppress symptoms and bacterial growth within an infected plant. These two sequences are now moving forward into the dual construct project described in the report entitled "Transgenic rootstock-mediated protection of grapevine scion by single and stacked DNA constructs".

Field Evaluations since September 2015 through February 2016: Plants have been dormant since the Fall of 2015. The plants are just beginning to bud as of March 15, 2016. All plants will evaluated for live-dead bud symptoms and samples taken for PCR analysis of bacterial presence on symptomatic and non-symptomatic inoculated buds and canes by the end of March.

RESEARCH RELEVANCE

The objective of this field experiment is to evaluate transgenic grape and grape rootstocks expressing various transgenes for protection against *Xylella fastidiosa* (Pierce's Disease strain) in a field site in Solano County. The pathogen is introduced into individual vines by mechanical injections of *X. fastidiosa* (*Xf*) into the grape stems of transgenic and non-transgenic control plants. The experiment is now in the fifth year after inoculations were initiated. Test plants include own-rooted transgenic and non-transgenic plants and grafted plants with non-transgenic scions of a PD susceptible variety grafted to root stocks bearing transgenes. The plants have been maintained under optimum field conditions with respect to water management, powdery mildew and insect control. Following the third (2013) and fourth (2014) years after inoculations began, control plants are showing clear symptoms of PD and many inoculated canes are dying or dead. These results were extended with a new inoculation in 2015 with comparable results as in 2014 but at this point most all of the control plants were dead in contrast to a number of the transgenic plants that remained healthy. The conclusion at this point is that several lines bearing the PR1 and UT456 DNA sequences effectively suppress PD symptoms, including some of the grafted plants. These two sequences are now moving forward into the dual construct project described in the report entitled "Transgenic rootstock-mediated protection of grapevine scion by single and stacked DNA constructs".

LAYPERSON SUMMARY

Previously, we identified novel genes that suppress PD symptoms by blocking programmed cell death (PCD), elicited by *Xf* through use of a functional screen from cDNA libraries of grape and tomato. Two of these sequences (PR1 and UT456) expressed as transgenes in grape, suppressed Pierce's Disease (PD) symptoms and dramatically reduced bacterial titer in inoculated plants under greenhouse conditions. Field experiments underway in Solano County, conducted with an APHIS permit, are designed to evaluate clonal copies of several of these transgenic lines under field conditions for resistance to PD. The field evaluation includes mechanical inoculation with *Xylella fastidiosa* in Solano County. Data sets include visual monitoring of plant morphology, PD symptoms and bacteria titer by quantitative PCR (qPCR) assays. To date, PCR data and plating assays confirm the presence of *Xf* in the plants. Inoculated untransformed plants are now showing typical symptoms of PD. Bacteria are present in inoculated plants at the Solano site and there is definitive evidence of symptom differences between several of the

transgenic lines compared with the non-transgenic control. Evaluation at the Solano County site are ongoing and inoculations continued in 2015.

FUNDING AGENCIES AND STATUS OF FUNDS: Funding for this project was provided by the California Department of Agriculture's Pierce's Disease Control Program under award 12-0443-SA and by the Regents of the University of California. All funds allocated to this project are expected to be expended by the end of the funding period that is June 30,2016.

REFERENCES

1. Gilchrist, David and James Lincoln 2011. Disease control and bacterial population dynamics in winegrape varieties grafted to rootstocks expressing anti-apoptotic sequences. Proceedings of the Pierce's Disease research symposium.
2. Gilchrist, David and James Lincoln 2012. 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.
3. Gilchrist, David and James Lincoln 2014. Field evaluation of grape plants expressing PR1 and UT456 transgenic DNA sequences for protection against Pierce's Disease. Proceedings of the 2013 Pierce's Disease Research Symposium

Figure 1 . Vine Pruning. As of March 15th, 2015, all plants were pruned to remove excess growth from the past year but to retain all inoculated 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.

Figure 2 Example of inoculated non-transgenic Thompson Seedless control plant with bud death as they emerge in spring 2015. Plant subjected to successive inoculations in 2011-2014. Xylella infection confirmed by PCR. Photo taken April 2, 2015. Colored tags visible on branches reflect the year and date of inoculation.

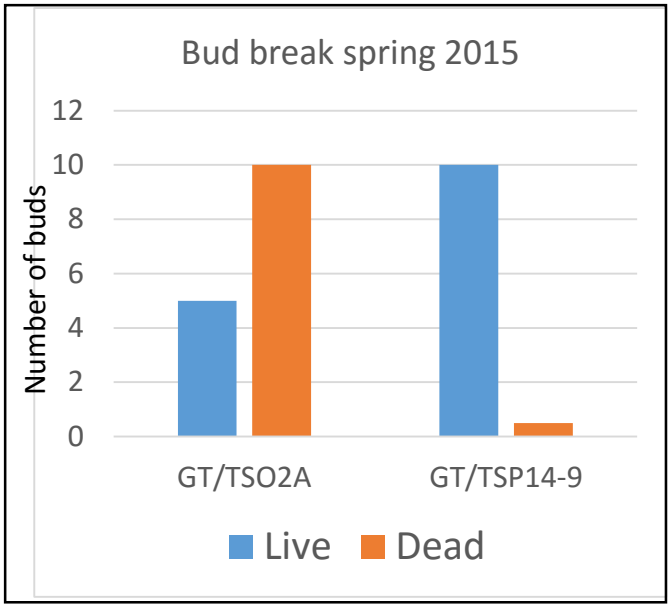

Figure 3. Live/Dead analysis at bud break on grafted plants. The graph indicates the number of buds from the designated genotypes on canes inoculated Spring 2014. All grafted plants have untransformed Thompson seedless as scions. GT/TSO2A is Thompson seedless untransformed as a rootstock. GT/TSP14-9 is Thompson seedless a rootstock expressing PR1.

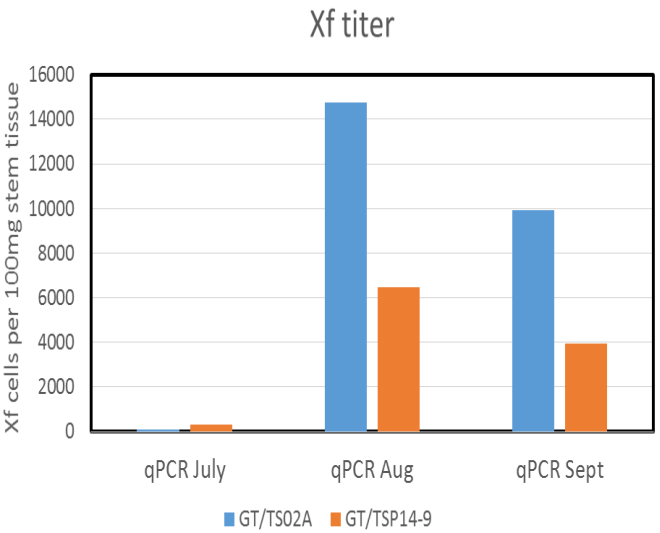

Figure 5. qPCR assessment of *Xylella fastidiosa* in untransformed Thompson Seedless scions grafted to untransformed Thompson Seedless rootstocks or transformed rootstocks. GT/TSO2A is Thompson seedless untransformed as a rootstock. GT/TSP14-9 is Thompson seedless expressing P14 as a rootstock. The respective untransformed Thompson Seedless scions were inoculated with *Xylella fastidiosa*.

Figure 4. Close up of non-transgenic control plant showing shoot dying shortly after emergence on inoculated canes in spring 2015. This cane was inoculated in 2013. Photo taken April 2, 2015

Figure 6. Inoculated non-transgenic Thompson Seedless foreground; essentially dead. Inoculated transgenic Thompson Seedless in the rear: asymptomatic. Image taken in May 2015. Tags indicate sites of inoculation.

Figure 7. Inoculated transgenic and non-transgenic Thompson Seedless seen from a distance. Asymptomatic and dying plants can be seen in the center row (arrow). May, 2015