Interim Progress Report 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.

Principal Investigator (PI) David Gilchrist; (530)752-6614. Department of Plant Pathology, Univ. of California, Davis, CA 95616. (dggilchrist@ucdavis.edu)

Co-Principal Investigator (Co-PI) James Lincoln; Department of Plant Pathology, Univ. of California, Davis, CA 95616. jelincoln@ucdavis.edu

Cooperator Mike Eldridge, (530) 754-7763. Field Supervisor Armstrong Research Field Area, Department of Plant Pathology

Time period covered by the report December 2014 to March 2015

Project History Initiated in 2010 with continuation through 2016.

INTRODUCTION

Field experiments were initiated in Solano County to 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*). Mechanical inoculation of *Xf* was employed at the Solano site. The Solano field experiment was conducted in two phases. 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. Data collected in 2012-14 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 (figure 1) As of March 15 2015, the plants have been pruned to remove excess dormant branches 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 a data rich resources for scoring dead vs live buds on control and transgenic plants as new shoots begin to emerge. The 2014 inoculated canes also will be destructively sampled to determine the presence and concentration of Xf in the tissues after scoring the new buds.

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. Controlled mechanical inoculation of *Xf* is used at the Solano County site. 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 P14 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.

The Solano experiment evaluates transgenic grape plants and grape rootstocks/scion combinations expressing a single DNA construct, designated PR1 and UT456, in a field site in Solano County for resistance to the Pierce's Disease strain of *Xylella fastidiosa* (*Xf*) following mechanical inoculation. 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. Preliminary data suggests that there is across graft protection (figure 2).

RESULTS AND DISCUSSION

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 3 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.

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.

Disease ratings 2014 and plant status March 2015:

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. 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. These evaluations will be continued when the plants emerge from dormancy.

As of March 15 2015, the plants have been pruned to remove excess dormant branches (figure 4) 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 a data rich resources for scoring dead vs live buds on control and transgenic plants as new shoots begin to emerge. The 2014 inoculated canes also will be destructively sampled to determine the presence and concentration of Xf in the tissues after scoring the new buds. .Buds are just beginning push and the first few leaves are showing.

CONCLUSIONS

Xylella fastidiosa induces PD symptoms that result from activation of a genetically regulated process of programmed cell death. We have identified two DNA sequences from a cDNA library screen, which, when constitutively expressed in transgenic grapes suppress the death-dependent symptoms of PD and reduce the bacterial titer to a level found in PD resistant wild grapes. We identified six novel anti-PCD genes from cDNA libraries of grape and tomato. Two of these grape sequences expressed as transgenes in grape, suppressed PD symptoms and dramatically reduced bacterial titer in inoculated plants in full plant transgenics in controlled greenhouse studies. Similar results are being seen under field conditions. Current data from the Solano site suggests that protective sequences may function across a graft union to protect an untransformed and susceptible wild type scion, although this data is preliminary. Both the PR1 and UT456 expressing plants show suppression of symptoms and reduced bacterial counts. Individual plants within 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.

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.

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 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 will continue in 2015.

STATUS OF FUNDS. All funds budgeted for these projects will be expended at the end of the current funding cycle as proposed.

SUMMARY AND STATUS OF INTELLECTUAL PROPERTY. The grape plants containing the anti-PCD genes and the grafted rootstocks will require the use of several patented enabling technologies. Record of invention disclosures have been submitted to the UC Office of Technology Transfer. The research proposed reported herein will provide data on the activity and mechanism of action of the protective transgenes in grape relative to the presence, amount and movement of Xylella fastidiosa in the transformed and untransformed grape plants.

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.

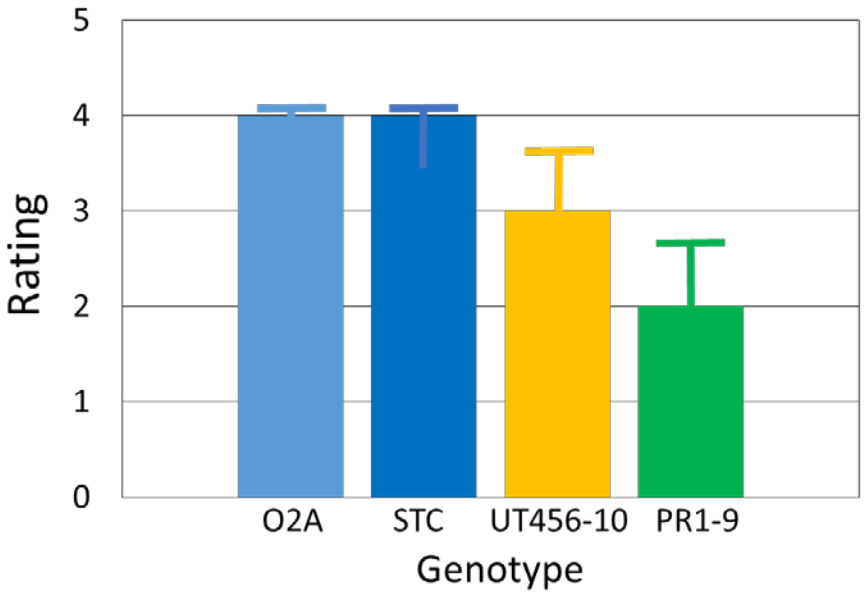

Figure 1. Evaluation of whole plant transgenics at the Solano site in summer of 2014. The susceptible controls are untransformed Thompson Seedless (O2A) and a susceptible transgenic line (STC), many plants within the controls were dead or dying at the end of the 2014 growing season. Individual plants within UT456 and PR1 lines have remained asymptomatic while some lines are less suppressive, all the transgenic lines are rated more suppressive of PD than the controls. There are no PD symptoms nor bacteria in the uninoculated susceptible controls. Rating scale is 0-5 with 0 being asymptomatic and 5 is a dead plant.

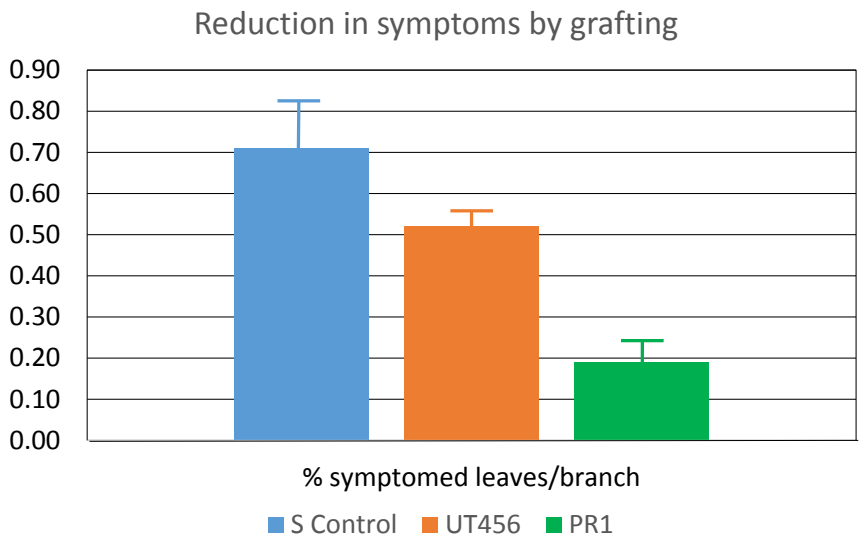

Figure 2. Evaluation of leaves on young shoots of a susceptible control plant grafted to untransformed (susceptible control) and transformed rootstocks expressing either UT456 or PR1 transgenes. Shoots were inoculated in Spring 2014; data taken Fall 2014

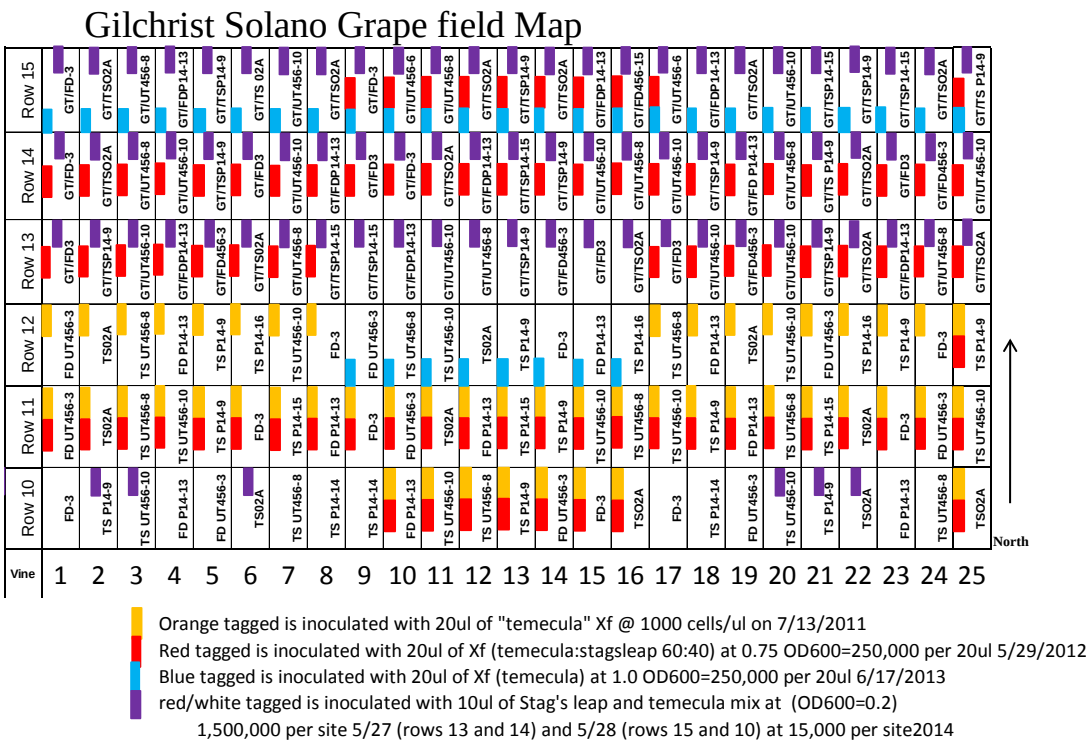

Figure 3. Map of Solano County field site. Genotypes of the plants are shown. GT/ is listed genotype grafted to Thompson Seedless scions. Inoculations were performed as indicated in 2011, 2012, 2013, and 2014.

Figure 4. Vine Pruning. As of March 15th, 2015, all plants have been 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.