Progress Report for CDFA Agreement Number 12-0443-SA

Project Title Field evaluation of grape plants expressing P14 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 (C0-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 March 2014 to July 2014

Project History Initiated in 2010 with continuation through 2016.

Introduction.

Field experiments were initiated in Riverside and Solano counties to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 for resistance to *Xylella fastidiosa* (Pierce's Disease strain) (Xf). Mechanical inoculation was employed at the Solano site whereas natural infection occurred at the Riverside site by endemic sharpshooters that carry Xf. 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. Controls in the first Solano site planting included own-rooted conventional Thompson Seedless and Freedom plants as controls to be compared with the transformed plants. Controls in the second phase at the Solano site consisted of, untransformed rootstocks grafted to the untransformed scions. Data collected in 2012-13 from both sites indicate that the bacteria are present in all plants at the Riverside site and in the mechanically inoculated plants at the Solano site (2).

Objectives of research ongoing

The objective is to continue to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated P14 and UT456 at the Riverside and Solano county sites for resistance to *Xf* (Pierce's Disease strain). Mechanical inoculation is used at the Solano County site and natural infection via the Glassy-winged Sharpshooter is the infection source at the Riverside site. The background research on selected transgenic lines leading to these field trials is from four inoculation experiments in a controlled greenhouse over a two year period, involving more than 300 transgenic plants of PR1and UT456. Each of these transgenes 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.

A. The first objective is to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 genes in a field site in Solano County for resistance to the Pierce's Disease strain of Xylella *fastidiosa* (*Xf*)

following mechanical inoculation. The first planting of fully transformed plants was 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 to assess for trans-graft protection against PD.

B. The second field experiment in Riverside County was planted in the spring of 2011. The planting consisted of clonal copies of the fully transformed ungrafted plants expressing PR1 or UT 456 that were planted in 2010 in Solano County. These Riverside plants will not be inoculated with *X. fastidiosa* but have been exposed to infection via natural populations of the Glassy-winged sharpshooter Xf vector.

Description of activities and results 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 are fertile.

Solano Planting:

As of May 2014, all inoculated plants at the Solano site are confirmed to harbor the introduced *Xf*. Uninoculated individuals are healthy, growing normally and tested free of *Xf* (Figure 1). The plants were confirmed to have been successfully infected in the 2011, 2012 and 2013 inoculations by sampling individual inoculated canes followed by qPCR analysis for relative bacterial populations (Figure 3). Bacterial numbers varied from 500-1500 cells per 1 cm of inoculated stem tissue. On May 28, 2014, 3-4 young shoots were inoculated with *Xf* in each of the plants by all investigators, including other the grafted and non-grafted plants expressing PR1 and UT456 in our specific set of plants (Figure 2). Evaluation of this set of inoculations will be done in late summer.

We observed at bud break in March of 2014 that excellent definitive differences were present in the form of dying buds and very young shoots that died quickly after emergence on 2011, 2012,or 2013, inoculated canes (now essentially cordons). Figures 4 and 5) shows an example of bud and shoot death only on previously inoculated canes, which was confirmed by detecting the presence of the bacteria by quantitative PCR (qPCR) assays in the inoculated canes. Hence, we conclude that evaluation of bud health in the spring is an important criteria to reveal the presence of sufficient bacteria in perennial tissues to cause serious disease and death (Figure 8).

By late June of 2014 all the inoculated untransformed control plants show foliar symptoms of PD (Figure 6), along with some of the experimental. 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. At this stage it is clear that there is a rich source of additional data to be collected that will prove important as we move forward in experiments now ongoing to combine (stack) the best of the transgenes into commercially accepted rootstocks. For future reference, the transgenic rootstocks will be grafted to untransformed scions to assess trans-graft protection of pairs of genes where the genes in the pairs will express different mechanisms of action, either acting as a bactericide, suppressing bacterial movement, blocking plant cell death or a quorum sensing modification of the bacteria.

Riverside Planting

Test plants were introduced to the field site in Riverside County with a history of severe presence of the Glassy-winged sharpshooter carrying Xylella fastidiosa in the spring of 2011. Sampling for presence of the bacteria in these plants confirmed widespread infection in the fall of 2011 but there was no evidence of PD symptoms at that time. Over the three years since planting, the plants

remained unthrifty and many began to die in the summer of 2012. Classical foliar symptoms of PD were rarely observed, especially in comparison to the mechanically inoculated plants in the Solano experiment. Initially, the plants expressing PR1 and UT456 transgenes were rated as more healthy that the non-transgenic controls. By the summer of 2013, it was clear that all the living plants, transgenic and non-transgenic were not growing normally and many had dead or dying cordons. Again, there was a absence of typical foliar PD symptoms on the canes that were clearly dying. In the spring of 2014, most all of the aerial portions of the plants, transgenic and non-transgenic were dead, although, suckers were emerging from base of many of these plants (Figure 7).

Coincidentally, the Dandekar planting directly adjacent to the Gilchrist plants also were all dead by the spring of 2014, with only random plants showing emergence of suckers. The Dandekar laboratory conducted extensive evaluation of the roots of the dead plants and confirmed extensive colonization by root-knot nematodes. The conclusion is that infestation by the root-knot nematode both confounded any data interpretation relative to PD and was the cause of the premature death of many , if not all, the plants. In addition, the foliar portions of all plants with living tissue from the suckers were aggressively pruned by the Riverside field crews in the summer of 2014 without informing any of the PIs with plants in the field. So, even if there were foliar symptoms of PD on the regrowth sucker branches, the pruning removed them and no data was collected. In summary, the Riverside planting was not useful in evaluating the transgenic plants of any of the investigators for response to PD over the period that plants were in this field. The lack of information was due primarily to the confounding impact of the root-know nematode and general unthriftyness of the plants from the outset (Figure 7).

Conclusions:

Xylella fastidiosa induces PD symptoms that result from activation of a genetically regulated process of programmed cell death. We have identified grape DNA sequences, which when constitutively expressed in transgenic grapes suppress the death-dependent symptoms of PD and reduce the bacterial titre to a level found in PD resistant wild grapes. We identified six novel anti-PCD genes from cDNA libraries of grape. 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. Initial data from the Solano site suggest that protective sequences may function across a graft union to protect an untransformed and susceptible wild type scion, although this data is very preliminary. This project has identified a basis for PD symptoms and a genetic mechanism to suppress symptoms and bacterial growth within an infected plant. Both the PR1 and UT456 expressing plants show suppression of symptoms and reduced bacterial counts. The plan for the coming year is to continue the field evaluation of transgenic grapes expressing PR1 and UT456 and to test for cross-graft protection by these two sequences, also under field conditions. In addition, we have initiated a project to stack these genes in pairs, along with those found to effective under field conditions by other researchers into commercially desired rootstocks.

Publications in preparation:

1. Juan Sanchez, James Lincoln, and David Gilchrist, 2014. Pathogenesis-related protein PR-1 interferes with programmed cell death and is synthesized under translational control
2. Juan Sanchez, James Lincoln, and David Gilchrist, 2014. The translation of pathogenesisrelated-PR-1 is triggered by a miRNA excised from grape coding sequences and the coding sequence of grape fanleaf virus.

3. James Lincoln and David Gilchrist. 2014. Pierce's Disease suppression in grape by transgenic expression of DNA sequences capable of blocking programed cell death.

Layperson summary of project accomplishments

Previously, we identified novel genes that suppress PD symptoms by blocking programmed cell death (PCD) by a functional screen from cDNA libraries of grape. Two of these grape 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 and Riverside counties, conducted with an APHIS permit, are intended to evaluate clonal copies of transgenic plants under field conditions for resistance to PD. The field evaluation was designed to involve mechanical inoculation with *Xylella fastidiosa* in Solano County and Glassy-winged Sharpshooter inoculation in Riverside 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 at both locations. Differential protection against defoliation was observed initially at the Riverside site and PCR assays confirmed bacterial populations in the plants, although the plants were generally unthrifty from the outset. However, by 2013-14 all plants at the Riverside site are dead or nearly dead but have not shown extensive foliar symptoms of PD. Recently, it has been discovered that the root systems of the plants are infected with the root-knot nematode presenting a serious confounding with any bacterial infection. Conversely, plants the Solano site remain vigorous with normal phenotypes. 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 plants and the non-transgenic control.

Research Timetable Two years from 7/1/14 to 6/30/16 Evaluation will continue through the June of 2016 at which point the planting will be destroyed following the protocol in the APHIS permit.

Research Capacity and Likelihood of Accomplishing Objectives The field area has been designated as an APHIS approved site under #7CFRE340. Commercialization of the currently effective anti-PCD containing vines and/or all experimental protocols have been used successfully to evaluate the field plants over the past 2 years and will be expected to continue to 2016.

Status of funds. Funds are being expended at a rate consistent with the proposed research plan.

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. David. Gilchrist, 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. Sacramento, CA December 13-15.
2. David. Gilchrist, and James Lincoln 2013. Field evaluation of grape plants expressing PR1 and UT456 transgenic DNA sequences for protection against Pierce's Disease. Proceedings of the 2013 Pierce' Disease Research Symposium

Figure 1. Solano County grafted grapes June 2014, trimmed to expose the tagged inoculation sites and permit sun exposure to the inoculated branches.

Figure 2. Young shoots of grafted plants were mechanically inoculated May 2014.

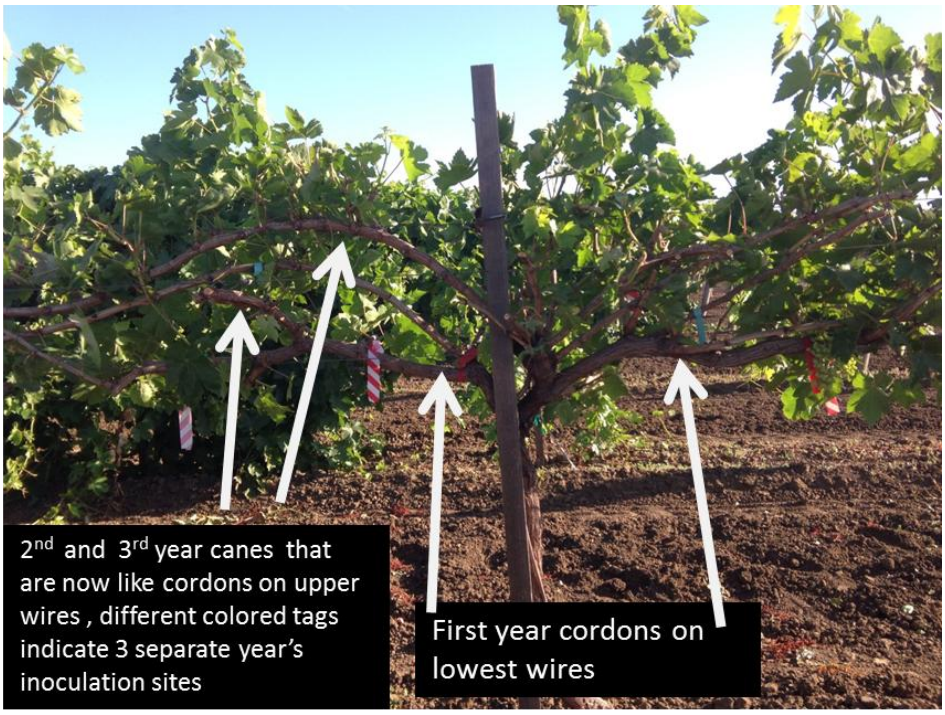

Figure 3. Illustration of successive cordons retained from successive inoculations done in 2011-2014. Photo taken late June 2014.

Figure 4. Illustration of bud and shoot death in the spring of 2014 due to Pierce's Disease on cordons inoculated in 2011 and 2012. Untransformed control Thompson Seedless (O2A), a susceptible transgenic control (STC) plant showing shoot death shortly after emergence compared with transgenic PD suppressive expressing difference anti-programmed cell death.

Figure 5. Close-up of spring PD symptoms where buds or very young shoots die shortly after emergence.

Figure 6. Illustration of foliar symptoms on young shoots of a susceptible control plant inoculated in spring of 2014.

Figure 7. Example of Riverside plants indicating that all aerial portions are dead with limited suckers emerging from the base of the plant, similar to those of the Dandekar lab, which are severely infected with root-knot nematode.

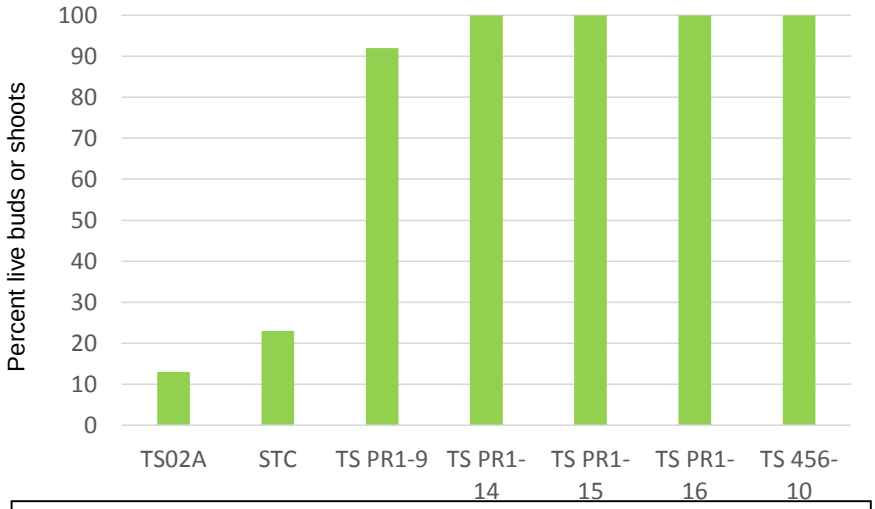

Figure 8. March 17, 2014 evaluation of inoculated canes on untransformed Thompson Seedless (TS02A), a transgenic susceptible control (STC) and transgenic lines expressing disease suppressive genes . 15 shoots were assessed in 3 plants per genotype as the plants emerged from dormancy. Figures 4 and 5 illustrate the stage of growth and appearance of the shoots