PROGRESS REPORT
CDFA 09-0780
March 2011 to July 2011

I. Project Title. Field evaluation of grape plants expressing PR1 and UT 456 transgenic DNA sequences for protection against Pierce's Disease

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III. OBJECTIVES
A. The overall 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 *Xylella fastidiosa* (Pierce's Disease strain) following mechanical inoculation.
B. The field experiments in Solano County will be conducted in two phases. The first phase of the field experiment starting in 2010 will evaluate clonal copies of the fully transformed ungrafted **PR1 and UT 456** plants that exhibited suppressed PD symptoms and low bacterial titers. There experiment will consist of inoculated sets and uninoculated control plants. All plants to be inoculated will be infected by stem puncture with ~20,000 bacterial cells per inoculation site. Timing of inoculation will depend on growth of the plants but is expected to occur late spring of 2011.
C. The second phase of the Solano County field planting will begin in 2011 with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks.
D. The field experiment to be planted in Riverside County will begin in the Spring of 2011. The planting will consist of clonal copies of the fully transformed ungrafted plants expressing PR1 or UT 456 that were planted in 2010 in Solano County. These plants will not be inoculated with *X. fastidiosa* but will be exposed to infection via the glassy winged sharpshooter vector of the bacteria.
IV. Summary of major research accomplishments through July 2011 for each objective
Each of the objectives has moved at the rate projected and interim goals for this fiscal year will be met. Details of the progress to date are presented in the following section.

Progress by Objective:
A. *The first phase of the field experiment starting in 2010 will evaluate clonal copies of the fully transformed ungrafted **PR1 and UT 456** plants that exhibited suppressed PD symptoms and low bacterial titers (2010-2013)*
This phase took place as planned with the planting occurring on July 12, 2010. Plants were placed in plastic sleeves to protect against sunburn and wind damage. The young plants had all emerged from the sleeves within two months and appear to be growing normally (Figure 1). Selection of canes to form cordons were made in spring 2011. Test plants were planted in a complete randomized block design. Field maps were prepared prior to planting and each plant is labeled with a permanent metal tag. Evaluation of the experimental plants for plant morphology, symptoms of Pierce's Disease infection, and the presence of the bacteria will be a time course

evaluation by visual monitoring of symptom development and sampling inoculated tissue for bacteria plant tissues (mainly leaves and stems) by quantitative PCR (qPCR) assays.

B. *A comparative quantitative determination by qPCR of the presence of Xylella in transgenic grape and grape rootstocks compared with conventional grape and grape rootstocks will provide an indication of the level of resistance to Pierce's Disease infection and the impact on the bacterial load in the respective transgenic and control plants.* All plants survived the winter and appear normal in appearance. On July 13, 2011, the Solano field plants were mechanically inoculated with 20,000 Xf "Temecula" bacteria cells (Figure 2). Fifty-one plants were inoculated and 24 plants will be used as uninoculated controls.

C. *The second phase of the Solano County field planting will begin in 2011 with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks (2010-2013).* Transgenic rootstocks for grafting were made by removing green shoots from greenhouse-grown plants of Thompson Seedless and Freedom expressing either PR1 or UT456, surface sterilized for 30 seconds in 70% ethyl alcohol, followed by 1% sodium hypochlorite solution containing 0.2% Tween 80 for 20 min with shaking, on a rotary shaker (50 rpm). The surface sterilized shoots are cut into single node pieces and placed into solid growth media to stimulate root formation. All the grafting is conducted in sterile Magenta GA-7 Plant Culture Boxes (3 x 3 x 4") containing 50 ml media under a 16 h light, 8 h dark photoperiod at 25$_{o}$C. Rootstock plantlets obtained *in vitro* are allowed to grow until several leaves are produced (4-6 weeks) and divided into 3–4 explants, each containing a single node. A scion with a single node and a leaf was selected to match the size of the rootstock, cut into a wedge to match a cleft made in the rootstock and was carefully fitted on to the cleft of the rootstock on the medium. After 4 weeks incubation healing in a magenta box, the rooted plantlet is transferred to sterile soil, allowed to heal and then transferred to the greenhouse for assays. Success rate is greater than 95% using this procedure, is more space efficient relative to greenhouse grafting, can be done anytime of the year, and is more rapid than green grafting. The plants for the Solano County phase two planting have been successfully grafted, grown to a suitable size for transplanting to the field and 75 grafted grape plants were planted June 22, 2011.

D. *Establish a field planting in Riverside, County consisting of clonal copies of the fully transformed ungrafted **PR1 and UT 456** plants that were planted in Solano County on July 12, 2010. (2011-2013)*
Field space was prepared in Riverside County and 75 plants, equivalent to the individuals in the Solano location were transported under permit to Riverside on May 25, 2011 and planted on May 26, 2011.

V. Publications: None
VI. Presentations. None
VII. Research relevance.
The objective is to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 genes in a field site at UC Davis for resistance to *Xylella fastidiosa* (Pierce's Disease strain) following mechanical inoculation. In four inoculation experiments in a controlled greenhouse over a two year period, involving more than 300 transgenic plants of PR1 and UT456, the suppression of PD symptoms and reduction in bacterial

titer has been consistent. 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. The field experiments will be conducted in two phases. The first phase of the field experiment starting in 2010 will evaluate clonal copies of the fully transformed ungrafted plants that exhibited suppressed PD symptoms and low bacterial titers. The second phase will begin with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks. 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 to be compared with the transformed plants. Controls in the second phase will include, untransformed rootstocks grafted to the untransformed scions to be compared with equivalent combinations expressing the test genes grafted to untransformed PD susceptible scions.

VIII. LAYPERSON SUMMARY

Previously, we identified novel anti-PCD genes 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. This project will evaluate clonal copies of these same plants under field conditions for resistance to (PD). The field evaluation will be conducted in Solano and Riverside Counties and will include mechanical inoculation with *X. fastidiosa* in Solano County and, hopefully, natural infection by the Glassy Winged Sharpshooter in Riverside County. Data sets will include visual monitoring of plant morphology, PD symptoms and bacteria titer by quantitative PCR (qPCR) assays.

Figure 1. Transgenic grape plants growing in Solano field June 2011.

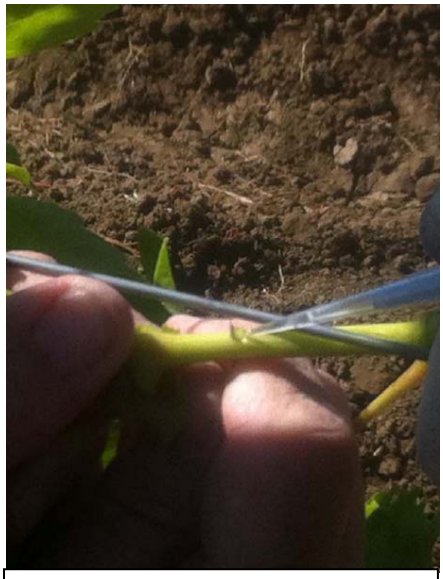

Figure 2. Mechanical inoculation of grape cane with 20,000 cells of Xf bacteria. Needle is inserted through cane from underneath. A 20ul drop containing the bacterial suspension is placed on needle tip and the needle is withdrawn. Negative pressure from transpiration pulls the drop quickly into the plant xylem.