Summary Final Report for CDFA Agreement number 16-0558-SA

Field Evaluation of Cross-Graft Protection Effective Against Pierce's Disease by Dual and Single DNA Constructs

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Reporting Period: This final report covers the period from 7/1/2016 to 6/30/2019

- **Introduction:** The project covers the completion of phase one and the beginning of phase two of field evaluation of DNA constructs integrated into grape plants to suppress the symptoms of Pierce's Disease (PD). The field evaluation, begun in 2010, has been supported with consecutive renewals to the present time and will continue with a new award covering the period July 1, 2019 through June 30, 2022.
- **Project Goal:** The project goal is to evaluate genetically modified plants bearing Pierce's Disease suppressive constructs under disease conditions in a USDA-APHIS regulated field environment. Initial field studies that began in 2010 tested five different single genes with different modes of action against PD. These constructs were integrated into two grape lines as whole plant transgenics (Thompson Seedless and Freedom rootstock). The data provided strong evidence that each of five genes protected but to differing levels of suppression of the symptoms of PD in cultivated grapes.
- **Objectives:** The objectives of grant period covered the final observations of the first planting, followed by the destructive removal of the first planting according to requirements of the APHIS agreement and re-tilling the entire field area. The area under Federal permit now includes a contiguous area that had not been planted previously and into which the new planting was begun in 2018 with new constructs developed in 2016-17.
- **Results:** This completed CDFA Agreement number 16-0558-SA established that untransformed control plants, mechanically injected with *X. fastidiosa* developed Pierce's Disease symptoms including both foliar and cane death symptoms within 24 months. The field tests further revealed positive protection against PD by five (5) different DNA constructs expressed as single constructs in a PD susceptible grape. A key result bearing on the integrity of the field research was that the bacteria did not move from plant to plant following inoculation thereby precluding any confounding of results from inoculated vs uninoculated control plants.
- **Ongoing Research:** A new planting has been established that consists of untransformed PD susceptible scions grafted to transgenic rootstocks (1103 and 110-14) expressing the paired constructs of the five genes. The field study will continue with support via a new award through June 30, 2022. The objective is to assess cross-graft protection of a non-transformed Chardonnay scion that is otherwise highly susceptible to Pierce's Disease. This research also will address the ability of the pathogenic bacteria to colonize and move within the xylem of the grape plant downward from the inoculated scion to the transgenic rootstock. The latter analysis will determine if the transgenic rootstock is differentially protected against Xylella induced death of the rootstock. The protocol includes planting, training, inoculating with *Xylella fastidiosa* to evaluate both disease and yield components in the untransformed Chardonnay scions in relation to uninoculated control plants and when grafted to untransformed rootstocks.