Interim Progress Report for CDFA Agreement Number 18-0306-000-SA

Project Title
Geographic distribution of isolate virulence in *Xylella fastidiosa* collected from grape in California and its effect on host resistance

Principal Investigator
Rachel P. Naegele  
USDA ARS  
Parlier, CA 93648

Co-Principal Investigator
Leonardo De La Fuente  
Auburn University  
Auburn, AL 36849-5413

Cooperator
Rodrigo Almeida  
UC Berkeley  
Berkeley, CA 94720

Time period covered by the report
7/01/2018 to 6/30/2019

Introduction
Plant pathogens with broad host ranges, like *Xf* if considered at the species level, often rely on multiple virulence and growth factors to colonize their diverse hosts. Though *Xf* was the first plant pathogenic bacterium to have its full genome sequenced, (7,14,15) only a small number of studies have looked at virulence variation (3,4,5,9,12,13). One small study in alfalfa, found significant correlation between genetic relatedness and virulence among 15 strains of *Xf* subsp. *fastidiosa* (3). In grape, virulence studies are lacking, but preliminary data suggest that virulence differences exist in CA. Further evaluation of virulence differences in *Xf* strains from CA is needed to understand the relationship between genetics and virulence.

Virulence comparisons among *Xf* strains are useful to understand the biology of this pathogen. In *Nicotiana tabacum* (tobacco), different subspecies of *Xf* are capable of colonizing and causing leaf scorch symptoms (1,10), and show differences in host colonization and symptomatology (6, 13). The co-PI De La Fuente used tobacco as a model system to understand changes in host mineral and nutrient composition caused by *Xf* infection (13), bacterial gene function (2,11), and impact of new DNA acquired from natural competence and recombination (8). The tobacco system will be used to determine the relative virulence of diverse strains and differential host response. Using tobacco to test multiple strains saves considerable greenhouse space and time, since a typical tobacco virulence experiment from planting to symptoms development takes ~ 3-4 months, half the time required for grapes.

PD resistance has been identified in multiple *Vitis* species. How these sources differ in durability (sustainability of resistance when exposed to multiple strains) of resistance is unclear. A single source of resistance *PDR1* from *V. arizonica*, a wild southwestern grape, has been used to develop high quality wine grapes with PD resistance (breeding efforts by Andy Walker, UC Davis). Table grape efforts also use this same source. Plants with *PDR1* have no disease symptoms and low bacterial populations when inoculated with *Xf*. *PDR1* has maintained efficacy in field trials in Texas and northern California, but its durability to multiple isolates is unclear.

List of objectives
Objective 1: Evaluate the virulence diversity of *Xylella fastidiosa* strains from California  
   Sub-objective a: Evaluate *Xf* isolate diversity in tobacco  
   Sub-objective b: Evaluate *Xf* isolate diversity in grape

Objective 2: Evaluate known grape sources of PD resistance against diverse strains of *X. fastidiosa*. 
Description of activities

Objective 1: Evaluate the virulence diversity of Xylella fastidiosa strains from California
  
  Sub-objective a: Evaluate Xf isolate diversity in tobacco
     - A graduate student has been hired to perform tobacco greenhouse evaluations.
     - Xylella fastidiosa isolates were received from Dr. Rodrigo Almeida and were propagated to ensure viability
     - Greenhouse inoculations have started using 13 strains plus Temecula and WM1-1 for control comparisons

  Sub-objective b: Evaluate Xf isolate diversity in grape
     - Field site has set up including new irrigation and metal stakes (not paid for with CDFA funds).
     - Cabernet Sauvignon plants (~ 900 vines) have been planted and pruned for Xylella inoculations.

Fig. 1. Cabernet Sauvignon 47 Uber vines planted on location at the SJV Agricultural Sciences Center

- Isolates of Xylella fastidiosa (~40) have been received from the collection of Dr. Almeida and are being propagated in the lab.

Objective 2: Evaluate known grape sources of PD resistance against diverse strains of X. fastidiosa.
  
  - Rooted plants from dormant cuttings previously collected are being maintained for known sources of PD resistance incl. 8909-08, Tampa, IAC 572, Bo25G, T56, B43-17, and BD5-117 in a growth chamber.
  - Green cuttings are being collected and propagated to increase plant numbers for the above genotypes. Additional sources of resistance are being propagated as a back up
in the event that sufficient numbers are not generated for the previously described list.

Fig 2. Green cuttings being propagated in a greenhouse

**Publications produced**
- Presented research updates to the CDFA PD/GWSS board at the December 2018 meeting (oral and poster), and wrote the accompanying proceedings paper
- No additional presentations have been made

**Relevance statement**
This project will provide the first look at the durability of host resistance to *Xylella fastidiosa* in the state of California, aiding breeders in developing resistant cultivars. In addition, this work will expand upon previous work looking at the genetic diversity of *X. fastidiosa* to determine if there are differences in the amount of disease that isolates from different areas can cause and its effect on management.

**Layperson summary**
Tobacco plants are currently being evaluated for susceptibility to *Xylella fastidiosa*. A test vineyard of Cabernet Sauvignon has been planted for artificial *Xylella* inoculations, which will take place in early July, at the San Joaquin Valley Agricultural Sciences Center.

**Status of funds**
Monies have been transferred to Auburn University. USDA has expended $493.70 of the received funds. These have been spent on petri dishes and media for *Xylella* propagation.

**Summary and status of intellectual property**
No intellectual property will be generated by this proposal.

**Literature cited**


