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	**Co-Principal Investigator**	**Cooperator**
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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

1. Alves, E., Kitajima, E.W., and Leite, B. (2003) Interaction of *Xylella fastidiosa* with different cultivars of *Nicotiana tabacum*: a comparison of colonization patterns. *Journal of Phytopathology-Phytopathologische Zeitschrift* **151**: 500-506.
2. Chen, H., Kandel, P.P., Cruz, L.F., Cobine, P.A., and De La Fuente, L. (2017) The major outer membrane protein MopB is required for twitching movement and affects biofilm formation and virulence in two Xylella fastidiosa strains. *Molecular Plant-Microbe Interactions*.
3. Coletta-Filho H.D., Bittleston L.S., Lopes J.R.S., Daugherty M.P., Almeida R.P.P. (2015) Genetic distance may underlie virulence differences among isolates of a bacterial plant pathogen. J Plant Path **97**:465-469.
4. Daugherty, M.P., Lopes, J.R.S., and Almeida, R.P.P. (2010) Strain-specific alfalfa water stress induced by *Xylella fastidiosa*. *European Journal of Plant Pathology* **127**: 333-340.
5. Francis, M., Civerolo, E.L., and Bruening, G. (2008) Improved bioassay of *Xylella fastidiosa* using *Nicotiana tabacum* cultivar SR1. *Plant Disease* **92**: 14-20.
6. Francis, M., Lin, H., Cabrera-La Rosa, J., Doddapaneni, H., and Civerolo, E.L. (2006) Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*. *European Journal of Plant Pathology* **115**: 203-213.
7. Janse, J.D., and Obradovic, A. (2010) *Xylella fastidiosa*: Its biology, diagnosis, control and risks. *Journal of Plant Pathology* **92**: S35-S48.
8. Kandel, P.P., Almeida, R.P.P., Cobine, P.A., and De La Fuente, L. (2017) Natural Competence Rates Are Variable Among *Xylella fastidiosa* Strains and Homologous Recombination Occurs In Vitro Between subspecies *fastidiosa* and *multiplex*. *Molecular Plant-Microbe Interactions* **30**: 589-600.
9. Lopes, J.R.S., Daugherty, M.P., and Almeida, R.P.P. (2010) Strain origin drives virulence and persistence of Xylella fastidiosa in alfalfa. *Plant Pathology* **59**: 963-971.
10. Lopes, S.A., Ribeiro, D.M., Roberto, P.G., Franca, S.C., and Santos, J.M. (2000) *Nicotiana tabacum* as an experimental host for the study of plant-*Xylella fastidiosa* interactions. *Plant Disease* **84**: 827-830.
11. Navarrete, F., and De La Fuente, L. (2015) Zinc Detoxification Is Required for Full Virulence and Modification of the Host Leaf lonome by Xylella fastidiosa. *Molecular Plant-Microbe Interactions* **28**: 497-507.
12. Oliver, J.E., Cobine, P.A., and De La Fuente, L. (2015) Xylella fastidiosa Isolates from Both subsp multiplex and fastidiosa Cause Disease on Southern Highbush Blueberry (Vaccinium sp.) Under Greenhouse Conditions. *Phytopathology* **105**: 855-862.
13. Oliver, J.E., Sefick, S.A., Parker, J.K., Arnold, T., Cobine, P.A., and De La Fuente, L. (2014) Ionome changes in *Xylella fastidiosa*-infected *Nicotiana tabacum* correlate with virulence and discriminate between subspecies of bacterial isolates. *Molecular Plant-Microbe Interactions* **27**: 1048-1058.
14. Simpson, A.J.G., Reinach, F.C., Arruda, P., Abreu, F.A., Acencio, M., Alvarenga, R. et al. (2000) The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature* **406**: 151-157.
15. Van Sluys, M.A., de Oliveira, M.C., Monteiro-Vitorello, C.B., Miyaki, C.Y., Furlan, L.R., Camargo, L.E.A. et al. (2003) Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. *Journal of Bacteriology* **185**: 1018-1026.