I. INTERIM PROGRESS REPORT FOR CDFA AGREEMENT NUMBER 15-0138-SA

II. TITLE OF PROJECT. Development of a biological control for Pierce's disease

III. PRINCIPAL INVESTIGATOR, CO-INVESTIGATORS, AND COOPERATORS.

PRINCIPAL INVESTIGATOR:
Thomas J. Burr
Dept. Plant Pathology & Plant-Microbe Biology
Cornell University/NYSAES
Geneva, NY 14456
tjb1@cornell.edu

CO-PRINCIPAL INVESTIGATOR(S):
Patricia Mowery
Dept. of Biology
Hobart & Wm. Smith Colleges
300 Pulteney St.
Geneva, NY 14456
mowery@hws.edu

Luciana Cursino
Dept. of Natural Sciences
& Mathematics
Keuka College
141 Central Ave.
Keuka Park, NY 14478
lparent@keuka.edu

Lingyun Hao
Dept. Plant Pathology & Plant-Microbe Biology
Cornell University/NYSAES
Geneva, NY 14456
lh459@cornell.edu

IV. TIME PERIOD COVERED BY THE REPORT. March 2016-July 2016

V. INTRODUCTION.

X. fastidiosa (*Xf*) is a Gram-negative, xylem-limited bacterium that causes Pierce's disease (PD) of grapevines (Chatterjee et al. 2008). *Xf* is transmitted to plants by insect vectors and once in the xylem, *Xf* is postulated to migrate, aggregate, and form biofilm that clogs the vessels leading to PD. We, and others, have studied *Xf* proteins and genetic mechanisms involved in these steps (Guilhabert and Kirkpatrick 2005, Meng et al. 2005, Feil et al. 2007, Li et al. 2007, Shi et al. 2007, da Silva Neto et al. 2008, Cursino et al. 2009, Cursino et al. 2011, Cursino et al. 2015) with the goal of better understanding PD virulence and for development of prevention strategies.

We deleted the *Xf* PD1311 gene (ΔPD1311), a putative acyl-CoA synthetase (ACS), as we were interested in genes potentially involved in secondary metabolite production. ACSs catalyze long-chain fatty acyl-CoAs (Black et al. 1992) and are involved in numerous processes including pathogenicity (Barber et al. 1997). We have just published our work on studying this gene, which includes showing it as having potential function as a biocontrol (Hao et al. 2016).

We found that PD1311 is a functional enzyme (data not shown), and that ΔPD1311 grows in PD2 and *Vitis vinifera* sap (**Fig.1)** (Hao et al. 2016). In addition, motility, aggregation, and biofilm production are key behaviors of *Xf* that are associated with PD (Chatterjee et al. 2008).

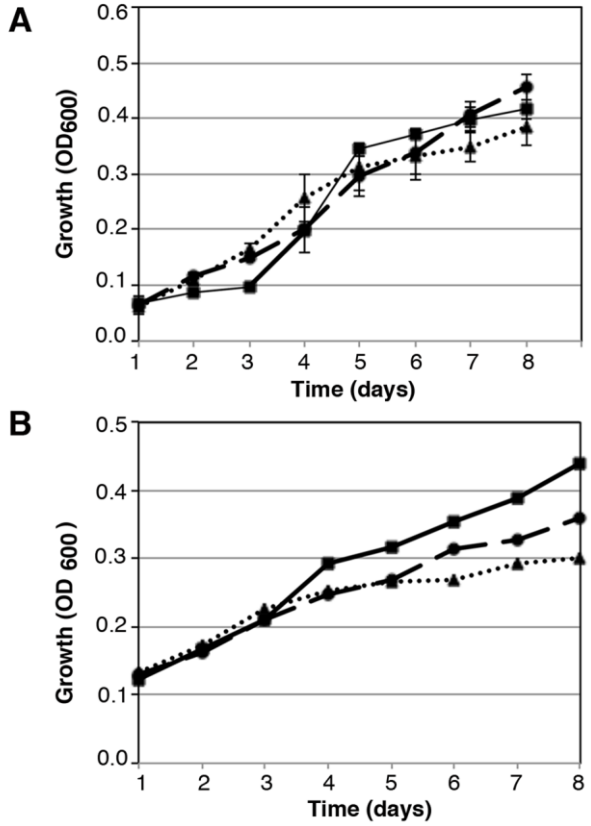

Fig. 1. ΔPD1311 growth and survival in grape sap. Shown are growth curves of TM1 (solid line, square), ΔPD1311 (dotted line, triangle) and C-ΔPD1311 (dashed line, circle) in PD2 broth (A) and 100% Chardonnay sap (B). Six replicates were included for each experiment and the assays were repeated three times. Error bars represent standard deviations. Three replicates were included for each experiment and the assay was repeated twice. TM1 = wild-type *X. fastidiosa* Temecula 1, ΔPD1311 = *X. fastidiosa* Temecula 1 deleted of the PD1311 gene, C-ΔPD1311 = ΔPD1311 complement strain.

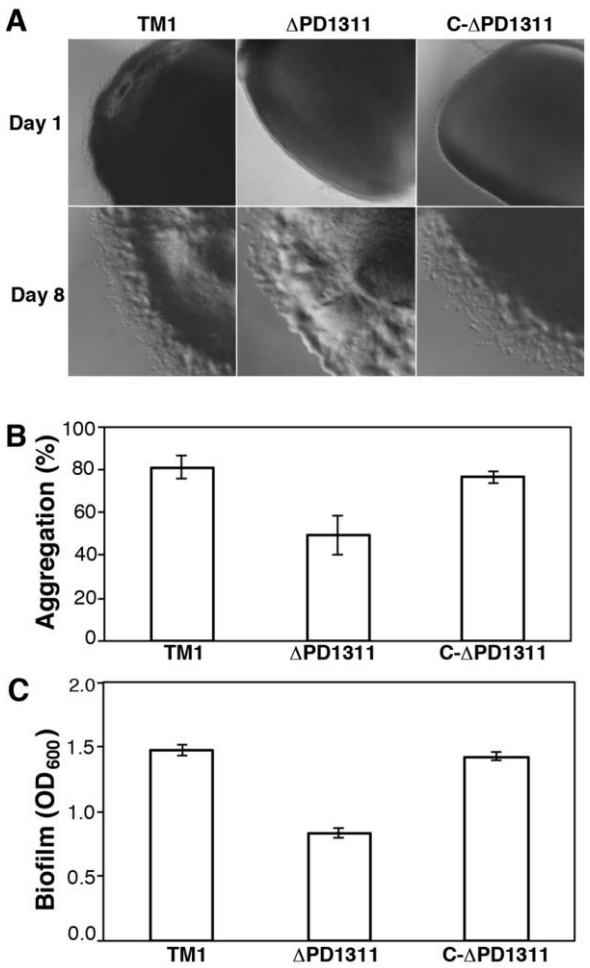

Fig. 2. ΔPD1311 was defective in motility, aggregation, and biofilm. A) Representative images of colony fringes of TM1, ΔPD1311 and C-ΔPD1311on PW-BSA plates at day 1 (top) and 8 (bottom) post-inoculation (p.i.). B) Mean percentage of aggregation and (C) biofilm quantification of wild-type, ΔPD1311, and C-ΔPD1311 strain in PD2 broth 5 d.p.i.. Error bars represent standard error. Twenty-four replicates were included for each experiment and the assay was repeated three times. * represents a significant difference of $p<0.01$. TM1 = wild-type *X. fastidiosa* Temecula 1, ΔPD1311 = *X. fastidiosa* Temecula 1 deleted of the PD1311 gene, and C-ΔPD1311 = ΔPD1311 complement strain.

ΔPD1311 is reduced in type IV pili-mediated motility on PW plates and is non-motile on sap agar (**Fig. 2**) (Hao et al. 2016). In comparison to wild-type cells (Temecula 1), ΔPD1311 is reduced in aggregation and biofilm production. We therefore hypothesized that ΔPD1311 is less virulent in plants, as mutants with similar phenotypes have been shown to have reduced or be avirulent (Cursino et al. 2009, Cursino et al. 2011, Guilhabert and Kirkpatrick 2005, Killiny et

al. 2013). We found that ΔPD1311 was avirulent and showed no PD, even at 24 weeks post-inoculation (**Fig. 3**).

The weakly virulent *Xf* elderberry strain EB92-1 has been studied as a potential PD biological control (Hopkins 2005, Hopkins 2012). Other approaches towards controlling PD include resistant rootstocks (Cousins and Goolsby 2011) and transgenic vines (Dandekar 2014, Gilchrist et al. 2014, Gilchrist and Lincoln 2014, Kirkpatrick 2014, Lindow 2014, Powell and Labavitch 2014). Continued research of PD controls is warranted. We had results that ΔPD1311 lowers the incidence of wild-type-induced PD. Given the avirulent phenotype of ΔPD1311 and its ability to limit wild-type induced PD, this strain provides new potential for a commercialized biological control

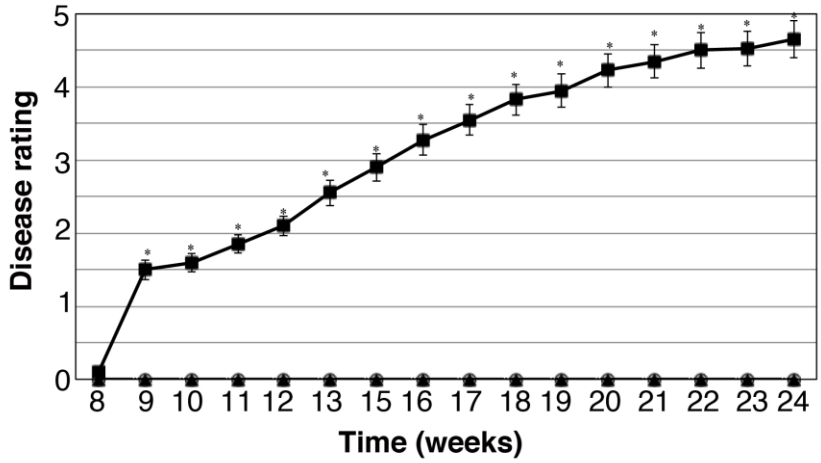

Fig. 3. ΔPD1311 is avirulent on grapevines. Shown are weekly mean disease ratings of vines inoculated with TM1 (solid line with squares), ΔPD1311 (triangles), C-ΔPD1311 (open circles) and buffer (dotted line on x-axis) respectively. Error bars represent standard errors. Ten plants were included for each experiment and the assay was repeated twice. * represents a significant difference of $p<0.01$. TM1 = wild-type *X. fastidiosa* Temecula 1, ΔPD1311 = *X. fastidiosa* Temecula 1 deleted of the PD1311 gene, C-ΔPD1311 = ΔPD1311 complement strain.

VI. LIST OF OBJECTIVES.

The overall goal is to optimize ΔPD1311 as a biological control for PD and to understand the mechanisms of disease inhibition that will facilitate commercialization.

Objective 1. Examine aspects of ΔPD1311 Temecula strain as a biological control of PD.

a. Optimize application timing and conditions for the ΔPD1311 strain.
b. Determine if over-wintered ΔPD1311 inoculated plants maintain PD resistance.
c. Explore leafhopper transmission of the ΔPD1311 strain.
d. Develop clean deletion strain of ΔPD1311 that would be suitable commercialization.

Objective 2. Determine the function of the PD1311 protein and the mechanism by which ΔPD1311 acts as a biological control.

a. Elucidate the role of PD1311 protein.
b. Examine impact of the ΔPD1311 strain on wild-type *Xf in vitro* and *in planta*.

VII. DESCRIPTION OF ACTIVIES.

Objective 1. Examine aspects of ΔPD1311 Temecula strain as a biological control of PD.

Objective 1a. Optimize application timing and conditions for the ΔPD1311 strain.

To examine if the *Xf* ΔPD1311 Temecula 1 strain could act as a potential biocontrol, we inoculated *V. vinifera* cv. Cabernet Sauvignon vines per standard procedures (Cursino et al. 2011) and recorded development of PD using the five-scale assessment (Guilhabert and Kirkpatrick 2005). We created three different inoculation conditions: i) wild-type *Xf* after a two-week pre-treatment with ΔPD1311 [following procedures used in *Xf* elderberry EB92.1 strain biocontrol studies (Hopkins 2005)], ii) wild-type and ΔPD1311 co-inoculated, and iii) controls (wild-type-only, ΔPD1311-only, buffer). We previously found that inoculating ΔPD1311 after a two-week pre-treatment with the wild-type strain did not limit PD (data not shown). Our controls included vines inoculated with wild-type Temecula 1, ΔPD1311, or buffer (Hopkins 1984). We found that pre-treatment with ΔPD1311 inhibits PD, while co-inoculation does not alter disease development (**Fig. 4**) (Hao et al. 2016).

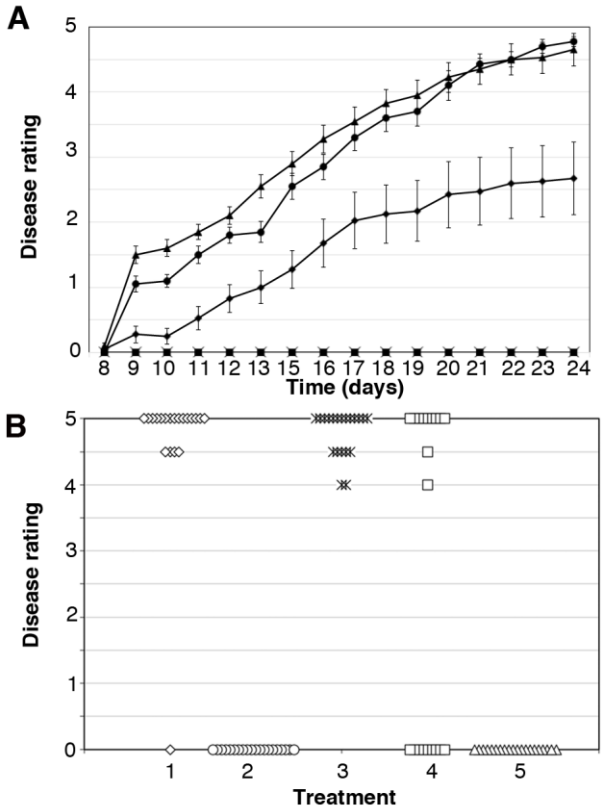

Fig. 4. ΔPD1311 inoculation to grape prior to TM1 suppressed PD development. A) Weekly mean disease ratings of vines inoculated with TM1-only (triangles), TM1 and ΔPD1311 simultaneously (circles), ΔPD1311 two weeks prior to TM1 (diamonds), ΔPD1311-only (squares) and buffer (x marks) respectively. Error bars represent standard errors. Ten plants were included for each experiment and the assay was repeated twice. B) Disease rating for each vine at 24 w.p.i. 1 = TM1-only, 2 = ΔPD1311-only, 3 = co-inoculation with TM1 and ΔPD1311 simultaneously, 4 = ΔPD1311 two weeks before TM1, and 5 = buffer. TM1 = wild-type *X. fastidiosa* Temecula 1, ΔPD1311 = *X. fastidiosa* Temecula 1 deleted of the PD1311 gene.

In 2016 summer, we are investigating the impact of ΔPD1311 pre-treatment timing and location on its biological control function. To test the impact of timing, we inoculated vines with ΔPD1311 at two days, one week, and two weeks (previous successful condition as described in **Fig. 4**) prior to inoculation with wild-type at the same inoculation point. To determine if inoculation location impacts PD control, we inoculated the base of selected green shoots (~ 50cm tall plant) with ΔPD1311 as described above and then two weeks later with wild-type *Xf* into vines at 5 or 30cm above the initial inoculation site. Our control treatments included the above treatments except with buffer instead of ΔPD1311, in order to exclude any possible effects on plants caused by wounding prior to wild-type. In addition, vines inoculated with wild-type-only, ΔPD1311-only, and buffer (Hopkins 1984) are also included as disease positive and negative controls. This experiment is underway.

Objective 1b. Determine if over-wintered ΔPD1311 inoculated plants maintain PD resistance.

In 2014 we had *V. vinifera* plants infected with wild-type *Xf* or ΔPD1311 two weeks prior to wild-type *Xf*. These vines were cut back and placed in nursery storage for the 2015 winter. The plants were then grown in the greenhouse in Spring 2015 to follow potential PD development. Preliminary results showed that wild-type *Xf* could overwinter and cause PD in the following year. Plants treated with ΔPD1311 followed by wild-type *Xf* did not show symptoms either year and ELISA did not detect *Xf* (Temecula 1 or ΔPD1311) in year 2 (**Table 1**). This data suggests that ΔPD1311 protection may last overwintering. However, we have not explored whether biocontrol treatment in year 1 would protect against a fresh wild-type inoculation in year 2. If found, this result would indicate that the ΔPD1311 biocontrol may have long-lasting protection in the field. If symptoms do develop in year 2 in the ΔPD1311-treated plants, this result will indicate that reapplication of the biocontrol will be necessary to maintain PD suppression.

Table 1. *Xf* ELISA results overwintered plants.[a]

Treatment Year 1[b]	Symptoms Year 1[c]	Symptoms Year 2[c]	0cm[de]	30cm[de]	150cm[de]
WT	+	+	+/1[e]	+/1	+/1
		-	-/3	-/3	-/3
ΔPD1311 then WT	-	-	-/2	-/2	-/2

[a] Plants overwintered in cold storage between year 1 and 2.
[b] Plants were given no further inoculations in year 2.
[c] "+" = PD symptoms; "-" = no PD symptoms.
[d] Sample distance up from inoculation point in year 2.
[e] "+"or "–" indicated positive or negative for *Xf*, respectively / "number" is the number of plants tested by ELISA in year 2.

The 2015-treated plants were stored in a cold-room overwinter. These included wild-type-only, ΔPD1311-only, ΔPD1311 two week before wild-type, and buffer-only plants. Half of the overwintered plants are being regrown without further treatment to determine if symptoms appear. The other half were allowed to grow for 1.5 months and then received new wild-type *Xf* inoculations at the base of the re-growing shoots. All plants are currently being followed for PD symptoms.

Objective 1c. Explore leafhopper transmission of the ΔPD1311 strain.

Xylem-sap feeding leafhopper vectors transmit *Xf* from plant to plant (Chatterjee et al. 2008). The bacterium utilizes adhesins, such as FimA, HxfA, and HxfB, to attach and form biofilms on insect foreguts, which then becomes a source of inoculum for further disease spread (Killiny and Almeida 2009, Killiny et al. 2010). Thus interaction with insects is a known key step for *Xf* to accomplish its life cycle. For development of ΔPD1311 as a commercially viable biological control agent and for future field studies, it will be necessary to

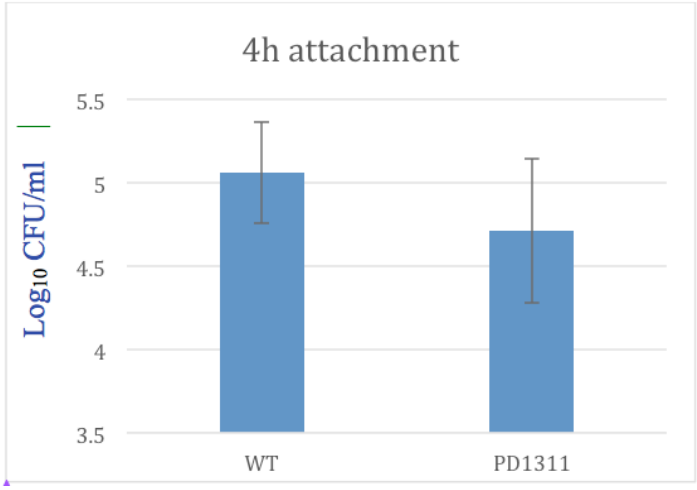

Fig. 5. The ΔPD1311 strain attached to leafhopper hind wings similarly to the wild-type strain. The attachment assay was performed as described previously (Baccari et al. 2014). The experiment was performed once with eight replicates included for each strain.

understand its insect transmissibility. Because ΔPD1311 has reduced aggregation and biofilm (**Fig. 2**), we hypothesize that ΔPD1311 is altered in its ability to be insect vectored. As an initial assay, we want to examine the adhesion of the mutant strain to the hindwing of the leafhopper vector, as this assay has been found to mimic adhesion to the foregut region owning to similar chitinous nature of the cuticles (Killiny et al. 2010). We have preliminary data to show that ΔPD1311 attached to insect wings at a level similarly to the wild-type strain (**Fig. 5**).

Objective 1d. Develop clean deletion strain of ΔPD1311 that would be suitable commercialization.

ΔPD1311 was created via site-specific recombination of a kanamycin cassette into the *Xf* chromosome (Matsumoto et al. 2009, Shi et al. 2009). For commercial viability, the antibiotic marker needs to be removed from the strain. Unlabeled *Agrobacterium tumefaciens* mutants have been created (Merritt et al. 2007), which will be the first approach we attempt. This work will begin after we complete data collection from objective 1a to confirm the biological control function of ΔPD1311 with optimized application conditions.

Objective 2. Determine the function of the PD1311 protein and the mechanism by which ΔPD1311 acts as a biological control.

Objective 2a. Elucidate the role of PD1311 protein.

The *Xf* PD1311 gene has motifs suggesting it encodes an ACS protein (acyl- and aryl-CoA synthetase) (Chang et al. 1997, Gulick 2009). ACS metabolite intermediates are involved in beta-oxidation and phospholipid biosynthesis. ACS proteins have also been implicated in cell signaling (Korchak et al. 1994), protein transportation (Glick and Rothman 1987), protein acylation (Gordon et al. 1991), and enzyme activation (Lai et al. 1993). Importantly, ACSs are involved in pathogenicity (Banchio and Gramajo 2002, Barber et al. 1997, Soto et al. 2002).

ACS proteins metabolize fatty acids through a two-step process to form a fatty acyl-CoA precursor utilized in any downstream metabolic pathways (Roche et al. 2013, Watkins 1997, Weimar et al. 2002). To confirm enzymatic activity, we expressed and purified a PD1311-His tag protein, and we tested it for ligase activity using acetate as the substrate. Acetate is the simplest substrate for fatty acid synthetase reaction, as a two-carbon (C2) chain length molecule. We used a standard colorimetric assay that measures acyl-CoA production (Kuang et al. 2007). The PD1311 protein exhibited a functional ATP/AMP binding domain that performed the following reaction: ATP + acetate + CoA is converted to AMP + pyrophosphate + acetyl-CoA (data not shown). Therefore we confirmed that the protein is functional.

The deletion of the PD1311 gene is non-lethal, suggesting that it has a role in non-essential fatty acid metabolism. One possibility is that PD1311 plays a role in DSF production, however, our preliminary results do not support that role (data not shown). An alternative potential role for the PD1311 protein is in precursor production of lipopolysaccharide (LPS). LPS is found on the outer membrane of gram-negative bacteria and is composed of a lipid A innermost component, a core saccharide, and an outer most O-antigen. Upstream of PD1311, are three genes annotated as LPS-associated enzymes: lipid A biosynthesis N-terminal domain protein (PD1312), dolichol-phosphate mannosyltransferase (Dpm1) (PD1313), and WbnF nucleotide sugar epimerase (PD1314) (Simpson et al. 2000). Dolichol-phosphate mannosyltransferase proteins are involved in N-linked oligosaccharides in the LPS core (Kapitonov and Yu 1999), while nucleotide sugar epimerases are involved in O-antigen synthesis (Lam et al. 2011). LPS is a known major virulence factor of *Xf*, and changes in LPS integrity renders bacteria more susceptible to environmental stress and defective in virulence (Clifford et al. 2013).

Considering the avirulent phenotype of ΔPD1311 on grapevines, PD1311 may be involved in lipid A biosynthesis or membrane production. Therefore, the ΔPD1311 cells may be more sensitive to environmental stresses such as oxidative stress and cationic antimicrobial peptide polymyxin B (PB). When wild-type and ΔPD1311 cells were exposed to hydrogen peroxide on agar plates in a Kirby-Bauer type assay, the zone of inhibition was greater for the mutant strain than wild-type cells (**Fig. 6A**) (Hao et al. 2016). In addition, ΔPD1311 cells were more sensitive to PB than wild-type or ΔPD1311 complement cells. While both wild-type and ΔPD1311 complement cells grew on plates supplemented with 16 µg/mL PB, almost all ΔPD1311 cells were killed when plated on PW agar supplemented with 1 ug/mL PB **(Fig. 6B).**

Objective 2b. Examine impact of the ΔPD1311 strain on wild-type Xf in vitro and in planta.

To have better grounding on why ΔPD1311 acts as a biological control, we need to explore the mechanism by which the mutant strain impacts wild-type cells. We have results showing that the wild-type induced disease can be limited only when ΔPD1311 was inoculated two weeks before the pathogen (**Fig. 4**). Therefore, we would like to know how the two strains spread through the plant when both are inoculated. ΔPD1311 does not secrete a toxin that affects wild-type populations (**Table 2**); we grew wild-type cells in supernatant from ΔPD1311 cells and found no growth changes (data not shown). Understanding how the mutant cells impact wild-type *Xf* is important for understanding not only how the biological control is achieved but also how the treatment would be most effectively applied in the field.

VIII. SUMMARY OF ACCOMPLISHMENTS AND RESULTS FOR EACH OBJECTIVE.

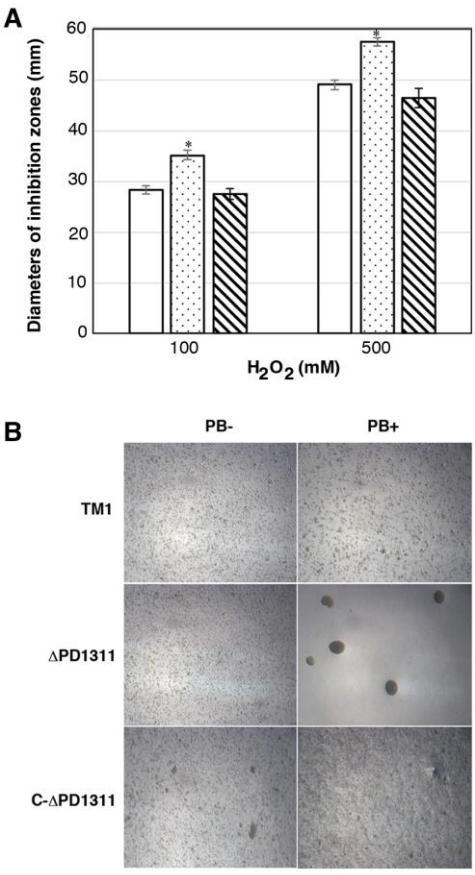

Fig. 6. Relative sensitivity of ΔPD1311 to H_2O_2 and polymixin B (PB). A) Mean diameters of inhibition zones of TM1 (empty bars), ΔPD1311 (dotted bars) and C-ΔPD1311 (dashed bars) exposed to 100 or 500 mM of H_2O_2 on PD2 agar plates. Error bars represent standard deviations. Three replicates were included for each experiment and the assay was repeated twice. * represents a significant difference of $p<0.01$. B) Growth of TM1 and C-ΔPD1311 on PD2 plates amended with 16 µg/mL PB and growth of ΔPD1311 on PD2 plates with 1 µg/mL PB. Images were taken under a Stemi-2000C dissecting microscope with a magnification of 3.2X. The assay was repeated at least four times with similar observations. TM1 = wild-type *X. fastidiosa* Temecula 1, ΔPD1311 = *X. fastidiosa* Temecula 1 deleted of the PD1311 gene, C-ΔPD1311 = ΔPD1311 complement strain.

Table 2. Wild-type *X. fastidiosa* detection by ELISA in petioles 24 w.p.i.[a]

Treatment	PD Symptom	Trial	Distance above inoculation point (cm)		
			0	30	150
ΔPD1311 then TM1[b]	-	1	-[c]/3[d]	-/3	-/3
		2	-/5	-/5	-/5
	+	1	n.d.[e]	n.d.	+/6
		2	n.d.	n.d.	+/4
TM1 + ΔPD1311	+	1	n.d.	n.d.	+/5
		2	n.d.	n.d.	+/4
TM1 only	+	1	n.d.	n.d.	+/5
		2	n.d.	n.d.	+/4

Shown are results of TM1 detection in petioles by ELISA 24 weeks post-inoculation. Each trial contained 10 plants total of which a subset was tested.

[a] w.p.i. = weeks post-inoculation.

[b] TM1 = wild-type; TM1 was inoculated two weeks after ΔPD1311.

[c] "+"or "–" indicates positive or negative for *X. fastidiosa*, respectively

[d] Number is the number of plants tested by ELISA.

[e] n.d. = not assessed as no petioles left due to disease.

Concerning objective 1, we confirmed that ΔPD1311 is avirulent, and we found that it can significantly reduce PD development by wild-type *Xf*. We are currently testing different inoculation locations to determine how infection position impacts disease protection. In addition, preliminary data suggests that ΔPD1311 attaches to insect hindwings equal to wild-type cells and therefore could possibly be distributed by the vector. We are completely the overwintering studies in objective 1b, which we hope will provide insights into the lasting impact of the ΔPD1311 biocontrol. For objective 2, our preliminary results show that the mutant has greater sensitivity to chemical environments (hydrogen peroxide, antimicrobial peptides), which may contribute to its avirulent phenotype and help explain the role of the protein in the bacterium. Much of our work in relation to this grant for has been recently published (Hao et al. 2016). Overall, this work will help further our understanding of disease development and prevention.

IX. PUBLICATIONS PRODUCED AND PENDING, AND PRESENTATIONS MADE THAT RELATE TO THE FUNDED PROJECT.

Publications (Peer reviewed and Proceedings).

Hao L, Zaini PA, Hoch HC, Burr TJ, Mowery P. 2016. Grape cultivar and sap culture conditions affect the development of *Xylella fastidiosa* phenotypes associated with Pierce's disease development. *PlosOne* in press.

Hao L, Johnson K, Cursino L, Mowery P, Burr TJ. 2016. Characterization of the *Xylella fastidiosa* PD1311 gene mutant and its suppression of Pierce's disease on grapevines. *Mol. Plant Pathol*. doi: 10.1111/mpp.12428.

Johnson KL, Cursino L, Athinuwat D, Burr TJ, Mowery P. 2015. Potential complications when developing gene deletion clones in *Xylella fastidiosa*. *BMC Res. Notes.* 8: 155.

Cursino L, Athinuwat D, Patel K, Galvani CD, Zaini PA, Li Y, De La Fuente L, Hoch HC, Burr TJ, Mowery P. 2015. Characterization of the *Xylella fastidiosa* PD1671 gene encoding degenerate c-di-GMP GGDEF/EAL domains, and its role in the development of Pierce's disease. *Plos One*. 10: e0121851.

Burr, T.J., Mowery, P., Cursino, L., and K. Johnson. Development of a biological control for

Pierce's disease. Proceedings of the Pierce's Disease Research Symposium 2015, pp. 9-17. Proceedings.

Burr, T.J., Mowery, P., Cursino, L., and K. Johnson. Development of a biological control for Pierce's disease. Proceedings of the Pierce's Disease Research Symposium 2014, pp. 42-49. Proceedings.

Burr, T.J., Mowery, P., Cursino, L., and Hao, L. Identification of a new virulence factor required for Pierce's disease and its utility in development of a biological control. Proceedings of the Pierce's Disease Research Symposium 2014, pp. 42-49. Proceedings.

Burr, T.J., Mowery, P., Cursino, L., and K. Johnson. Identification of a new virulence factor required for Pierce's disease and its utility in development of a biological control. Proceedings of the Pierce's Disease Research Symposium 2013, pp. 41-47. Proceedings.

Mowery, P., T.J., Burr, Hoch, H.C., Cursino, L., Johnson, K., Galvani, C., Athiuwat, D., and Shi, X. Exploiting a chemosensory signal transduction system that controls twitching motility and virulence in *Xylella fastidiosa*. Proceedings of the Pierce's Disease Research Symposium 2012, pp. 59-64. Proceedings.

Cursino, L., Galvani, C.D., Athinuwat, D., Zaini, P.A., Li, Y., De La Fuente, L., Hoch, H.C., Burr, T.J., and P. Mowery. 2011. Identification of an Operon, Pil-Chp, that Controls Twitching Motility and Virulence in *Xylella fastidiosa*. *Mol. Plant Microbe Interact.* 24:1198-1206.

Mowery, P., T.J., Burr, Hoch, H.C., Cursino, L., Athiuwat, D., and Galvani, C. Exploiting a chemosensory signal transduction system that controls twitching motility and virulence in *Xylella fastidiosa*. Proceedings of the Pierce's Disease Research Symposium 2011, pp. 71-75. Proceedings.

Presentations and Posters.

Mowery P. *Xylella fastidiosa* chemosensory-like involvement in Pierce's disease." American Society of Phytopathology, Pasadena, CA, 2015. Presentation.

Hao L, Johnson K, Cursino L, Burr TJ, Mowery P. *Xylella fastidiosa* PD1311 deleted strain as promising Pierce's disease biological control. American Society of Phytopathology, Pasadena, CA, 2015. Poster.

Burr TJ. PD1311, a virulence factor required for Pierce's disease and its utility in development of a biological control. Pierce's Disease Research Symposium, Sacramento, CA, 2014. Presentation.

Burr TJ. How *Xylella fastidiosa* is able to move in plants. Pierce's Disease Research Symposium, Sacramento, CA, 2013. Presentation.

Johnson K, Mowery P, Burr TJ. Impact of aggregation on development of *Xylella fastidiosa* mutant clones. Pierce's Disease Research Symposium, Sacramento, CA, 2013. Poster.

Mowery P, Johnson KL, Cursino L, Burr TJ. Identification of a new virulence factor required for Pierce's disease and its utility in development of a biological control. Pierce's Disease Research Symposium, Sacramento, CA, 2013. Poster.

Johnson K. Role of a thioredoxin family protein in *Xylella fastidiosa* virulence. APS-MSA, Austin, TX, 2013. Presentation.

Mowery P, Johnson KL, Cursino L, Burr TJ. *Xylella fastidiosa* virulence factor mutant strain as a potential biocontrol for Pierce's disease. APS-MSA, Austin, TX, 2013. Poster.

Mowery P. “How does your vineyard grow? Understanding the grapevine pathogen, *Xylella fastidiosa*.” Department of Biology. Ithaca College. Ithaca, NY, 2013. Presentation.

X. RESEARCH RELEVANCE STATEMENT.

Xylella fastidiosa is an important phytopathogen that infects a number of important crops including citrus, almonds, and coffee. The *X. fastidiosa* Temecula strain infects grapevines and induces Pierce’s disease. We recently deleted the *X. fastidiosa* PD1311 gene and found that the mutant strain is avirulent. Based on sequence analysis, PD1311 is predicted to encode an acyl-CoA synthetase, which is a class of enzymes involved in many different processes including secondary metabolite production. We have characterized ΔPD1311 and found phenotypes consistent with reduced virulence. In addition to ΔPD1311 being avirulent it also reduces the virulence of wild-type *X. fastidiosa*. Therefore, we propose that the ΔPD1311 has potential as a biological control for PD.

XI. LAY SUMMARY OF PROJECT ACCOMPLISHMENTS.

We discovered that deleting the *X. fastidiosa* Temecula 1 gene, PD1311, results in a strain that does not induce Pierce’s Disease. Additionally, we have evidence that the PD1311 mutant has potential as a biological control. When grape plants were inoculated with the mutant prior to wild-type *X. fastidiosa*, disease development becomes significantly reduced. Given the agricultural importance of Pierce’s Disease, it is critical to understand how PD1311 exerts its effects. Options for managing Pierce’s Disease are limited, which makes development of new control strategies critically important. The results from our aims expand our understanding of Pierce’s Disease and provide information in relation to controlling the disease.

XII. STATUS OF FUNDS.

$107,711.05 of the funds are left. We requested modification to the budget to have sufficient salary to conduct the experiments that are underway.

XIII. SUMMARY AND STATUS OF INTELLECTUAL PROPERTY ASSOCIATED WITH THE PROJECT.

No intellectual property has resulted from research done under this grant. However further development of the PD1311 mutant could result in a commercially viable control for PD.

XIV. LITERATURE CITED.

Baccari C, Killiny N, Ionescu M, Almeida RP, Lindow SE. 2014. Diffusible signal factor-repressed extracellular traits enable attachment of *Xylella fastidiosa* to insect vectors and transmission. *Phytopathology*. 104: 27-33.

Banchio C, Gramajo H. 2002. A stationary-phase acyl-coenzyme A synthetase of *Streptomyces coelicolor* A3(2) is necessary for the normal onset of antibiotic production. *Appl. Environ. Microbiol.* 68:4240-4246.

Barber CE, Tang JL, Feng JX, Pan MQ, Wilson TJG, Slater H, Dow JM, Williams P, Daniels MJ. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol. Microbiol.* 24:555–566.

Black PN, DiRusso CC, Metzger AK, Heimert TL. 1992. Cloning, sequencing, and expression of the *fadD* gene of *Escherichia coli* encoding acyl coenzyme A synthase. *J. Biol. Chem.* 267:25513–25520.

Burdman S, Jrukevitch E, Soria-Diaz ME, Serrano AMG, Okon Y. 2000. *FEMS Microbiol. Lett.* 189:259-264.

Chang KH, Xiang H, Dunaway-Mariano D. 1997. Acyl-adenylate motif of the acyl-adenylate/thioester-forming enzyme superfamily: a site-directed mutagenesis study with the *Pseudomonas* sp. strain CBS3 4-chlorobenzoate:coenzyme A ligase. *Biochemistry.* 36:15650-15659.

Chatterjee S, Almeida RPP, Lindow S. 2008. Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. *Annu. Rev. Phytopathol.* 46:243-271.

Clifford JC, Rapicavoli JN, Roper MC. 2013. A rhamnose-rich O-antigen mediates adhesion, virulence, and host colonization for the xylem-limited phytopathogen *Xylella fastidiosa*. *Mol. Plant Microbe Inter.* 26:676–685.

Cousins PS, Goolsby J. 2011. Grape rootstock variety influence on PD symptoms in chardonnay. In *Pierce's Disease Research Symp. Proc.*, p. 99.

Cursino L, Athinuwat D, Patel KR, Galvani CD, Zaini PA, Li Y, De La Fuente L, Hoch HC, Burr TJ, Mowery P. 2015. Characterization of the *Xylella fastidiosa* PD1671 gene encoding degenerate c-di-GMP GGDEF/EAL domains, and its role in the development of Pierce's Disease. *PlosOne*. 10:e0121851.

Cursino L, Galvani CD, Athinuwat D, Zaini PA, Li Y, De La Fuente L, Hoch HC, Burr TJ, Mowery P. 2011. *Mol. Plant Microbe Inter.* 24:1198-1206.

Cursino L, Li Y, Zaini PA, De La Fuente L, Hoch HC, Burr TJ. 2009. Twitching motility and biofilm formation are associated with *tonB1* in *Xylella fastidiosa*. *FEMS Microbiol. Lett.* 299:193-199.

da Silva Neto JF, Koide T, Abe CM, Gomes SL, Marques MV. 2008. *Arch. Microbiol.* 189:249-261.

Dandekar A.M. 2014. Chimeric antimicrobial protein and dpolygalacturonase-inhibiting protein transgenic grapevine field trial. In *Pierce's Disease Research Symp. Proc.*, pp. 106-117. Calif. Dep. Food Agric.

Davis MJ, Purcell AH, Thomson SV. 1980. Isolation media for Pierce's Disease bacterium. *Phytopathology.* 70:425-429.

Feil H, Feil WS, Lindow SE. 2007. Contribution of fimbrial and afimbrial adhesins of *Xylella fastidiosa* of attachment to surfaces and virulence to grape. *Phytopathology.* 97:318-324.

Gilchrist D, Dandekar A, Powerll A, Lindow S. 2014. Field evaluation of grape plants expressing potential protective DNA sequences effective against Pierce's Disease. In *Pierce's Disease Research Symp. Proc.*, pp. 122-125. Calif. Dep. Food Agric.

Gilchrist D, Lincoln J. 2014. Field evaluation of grape plants expressing PR1 and UT456 transgenic DNA sequences for protection against Pierce's Disease. In *Pierce's Disease Research Symp. Proc.*, pp. 126-132. Calif. Dep. Food Agric.

Glick BS, Rothman JE. 1987. Possible role for fatty acyl-coenzyme A in intracellular protein transport. *Nature.* 326:309-312.

Gordon JI, Duronio RJ, Rudnick DA, Adams SP, Gokel GW. 1991. Protein N-myristoylation. *J. Biol. Chem.* 266:8647-8650.

Guilhabert MR, Kirkpatrick BC. 2005. Identification of *Xylella fastidiosa* antivirulence genes: hemagglutinin adhesins contribute to *Xylella fastidiosa* biofilm maturation and colonization and attenuate virulence. *Mol. Plant Microbe Interact.*18:856–868.

Gulick AM. 2009. Conformational dynamics in the acyl-CoA synthetases, adenylation domains of non-ribosomal peptide synthetases, and firefly luciferase. *ACS Chem. Biol.* 4:811-827.

Hao L, Johnson K, Cursino L, Mowery P, Burr TJ. 2016. Characterization of the *Xylella fastidiosa* PD1311 gene mutant and its suppression of Pierce's disease on grapevines. *Mol. Plant Pathol*. doi: 10.1111/mpp.12428.

Hopkins DL. 2005. Biological control of PD in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. *Plant Dis.* 89:1348-1352.

Hopkins D.L. 2012. Biological control of Pierce's Disease of grapevine with benign strains of *Xylella fastidiosa*. In *Pierce's Disease Research Symp. Proc.*, pp. 125-128. Calif. Dep. Food Agric.

Kapitonov D, Yu RK. 1999. Conserved domains of glycosyltransferases. *Glycobiology*. 9:961-978.

Killiny N, Almeida RPP. 2009. *Xylella fastidiosa* afimbrial adhesins mediate cell transmission to plants by leafhopper vectors. *Appl Environ. Microbiol.* 75:521–528.

Killiny N., Martinez RH, Dumenyo CK, Cooksey DA, Almeida RP. 2013. The exopolysaccharide of *Xylella fastidiosa* is essential for biofilm formation, plant virulence, and vector transmission. *Mol. Plant Microbe Interact.* 26: 1044-1053.

Killiny N, Prado SS, Almeida RPP. 2010. Chitin utilization by the insect-transmitted bacterium *Xylella fastidiosa*. *Appl. Environ. Microbiol.* 76:6134–6140.

Kirkpatrick B. 2014. Evaluation of Pierce's Disease resistance in transgenic *Vitis vinifera* grapevines expressing *Xylella fastidiosa* hemagglutinin protein. In *Pierce's Disease Research Symp. Proc.*, pp. 141-148. Calif. Dep. Food Agric.

Korchak HM, Kane LH, Rossi MW, Corkey BE. 1994. Long chain acyl coenzyme A and signaling in neutrophils. An inhibitor of acyl coenzyme A synthetase, triacsin C, inhibits superoxide anion generation and degranulation by human neutrophils. *J. Biol. Chem.* 269:30281-30287.

Kuang Y, Salem N, Wang F, Schomisch SJ, Chandramouli V, Lee Z. 2007. A colorimetric assay method to measure acetyl-CoA synthetase activity: application to woodchuck model of hepatitis virus-induced hepatocellular carcinoma. *J. Biochem. Biophys. Meth.* 70:649-655.

Lai JCK, Liang BB, Jarri EJ, Cooper AJL, Lu DR. 1993. Differential affects of fatty acyl coenzyme A derivatives on citrate synthase and glutamate dehydrogenase. *Res Commun. Chem Pathol. Pharmacol.* 82:331-338.

Li Y, Hao G, Galvani CD, Meng Y, De La Fuente L, Hoch HC, Burr TJ. 2007. Type I and type IV pili of *Xylella fastidiosa* affect twitching motility, biofilm formation and cell-cell aggregation. *Microbiology.* 153:719-726.

Lindow SE. 2014. Continued field evaluation of diffusible signal factor producing grape for control of Pierce's Disease. In *Pierce's Disease Research Symp. Proc.*, pp. 162-171. Calif. Dep. Food Agric.

Matsumoto A, Young GM, Igo MM. 2009. Chromosome-based genetic complementation system for *Xylella fastidiosa*. *Appl. Enviorn. Microbiol.* 75:1679-1687.

Meng Y, Galvani CD, Hao G, Turner JN, Burr TJ, Hoch HC. 2005. Upstream migration of *Xylella fastidiosa* via pili-driven twitching motility. *J. Bacteriol*. 187:5560–5567.

Merritt PM, Danhorn T, Fuqua C. 2007. Motility and chemotaxis in *Agrobacterium tumefaciens* surface attachment and biofilm formation. *J. Bacteriol.* 189:8005-8014.

Powell ALT, Labavitch JM. 2014. Field evaluation of grafted grape lines expressing polygalacturonase-inhibiting proteins. In *Pierce's Disease Research Symp. Proc.*, pp. 172-179. Calif. Dep. Food Agric.

Roche CM, Blanch HW, Clark DS, Glass NL. 2013. Physiological role of acyl coenzyme A synthetase homologs in lipid metabolism in *Neurospora crassa*. *Eukaryot. Cell*. 12:1244–1257.

Shi XY, Dumenyo CK, Hernandez-Martinez R, Azad H, Cooksey DA. 2007. *Appl. Environ. Microbiol.* 73:6748-6756.

Shi XY, Dumenyo CK, Hernandez-Martinez R, Azad H, Cooksey DA. 2009. Characterization of regulatory pathways in *Xylella fastidiosa*: genes and phenotypes controlled by *gacA*. *App. Enivorn. Microbiol.* 75:2275-2283.

Simpson AJ, Reinach FC, Arruda P, Abreu FA, Acencio M, et al. 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature.* 406:151-157.

Soto MJ, Fernandez-Pascual M, Sanjuan J, Olivares J. 2002. A *fadD* mutant to *Sinorhizobium meliloti* shows multicellular swarming migration and is impaired in nodulation efficiency on alfalfa roots. *Mol. Microbiol.* 43:371–382.

Watkins P. 1997. Fatty acid activation. *Prog. Lipid Res.* 36:55-83.

Weimar JD, DiRusso CC, Delio R, Black PN. 2002. Functional role of fatty acyl-coenzyme A synthetase in the transmembrane movement and activation of exogenous long-chain fatty acids. *J. Biol. Chem.* 277:29369–29376.

Zaini PA, De La Fuente L, Hoch HC, Burr TJ. 2009. Grapevine xylem sap enhances biofilm development by *Xylella fastidiosa*. *FEMS Microbiol. Lett.* 295:129-134.