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.

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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).

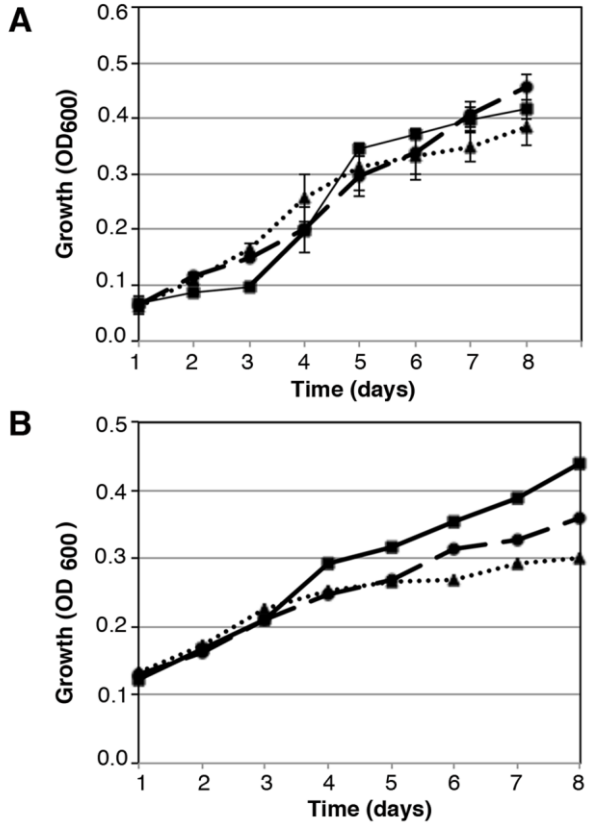


Fig. 1. ΔPD1311 growth and survival in grape sap. Shown are growth curves of TM1 (solid line, square), ΔPD1311 (dotted line, triangle) and C-ΔPD1311 (dashed line, circle) in PD2 broth (A) and 100% Chardonnay sap (B). Six replicates were included for each experiment and the assays were repeated three 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.

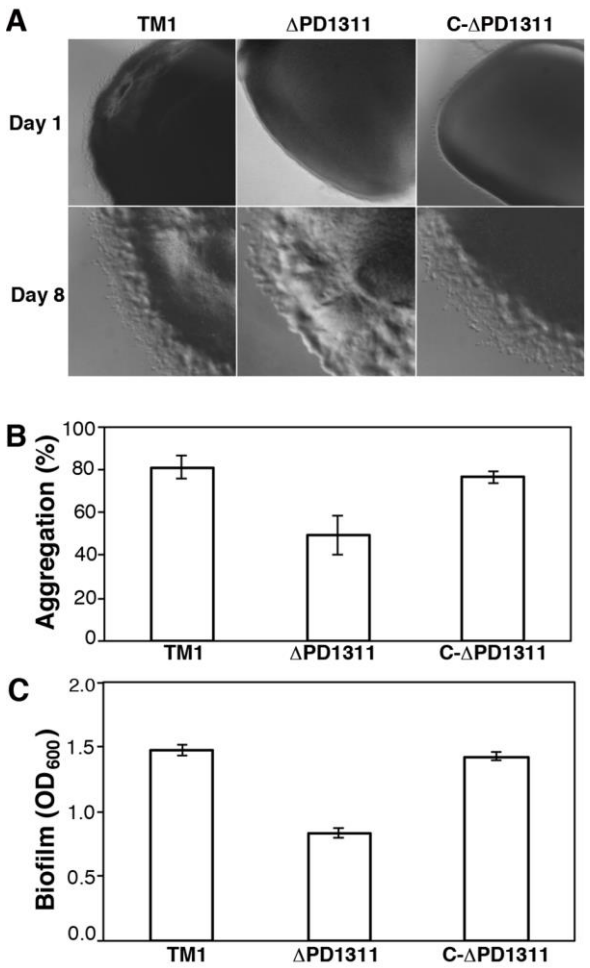


Fig. 2. ΔPD1311 was defective in motility, aggregation, and biofilm. A) Representative images of colony fringes of TM1, ΔPD1311 and C-ΔPD1311on PW-BSA plates at day 1 (top) and 8 (bottom) post-inoculation (p.i.). B) Mean percentage of aggregation and (C) biofilm quantification of wild-type, ΔPD1311, 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

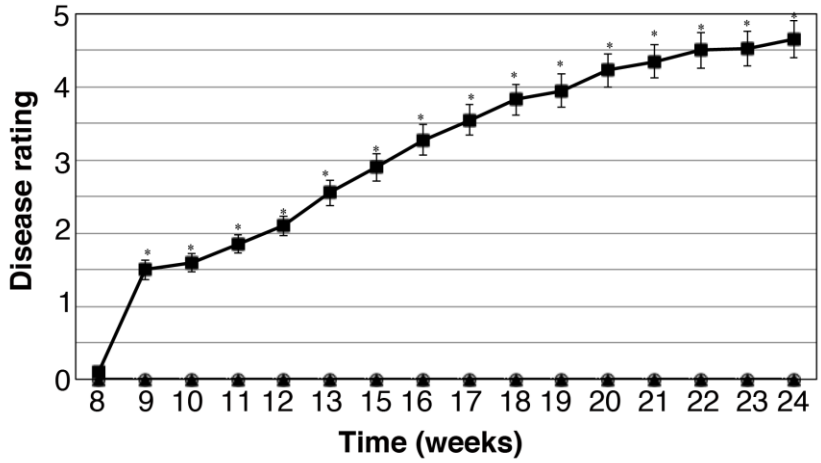


Fig. 3. ΔPD1311 is avirulent on grapevines. Shown are weekly mean disease ratings of vines inoculated with TM1 (solid line with squares), ΔPD1311 (triangles), C-ΔPD1311 (open circles) and buffer (dotted line on x-axis) respectively. Error bars represent standard errors. Ten plants were included for each 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).

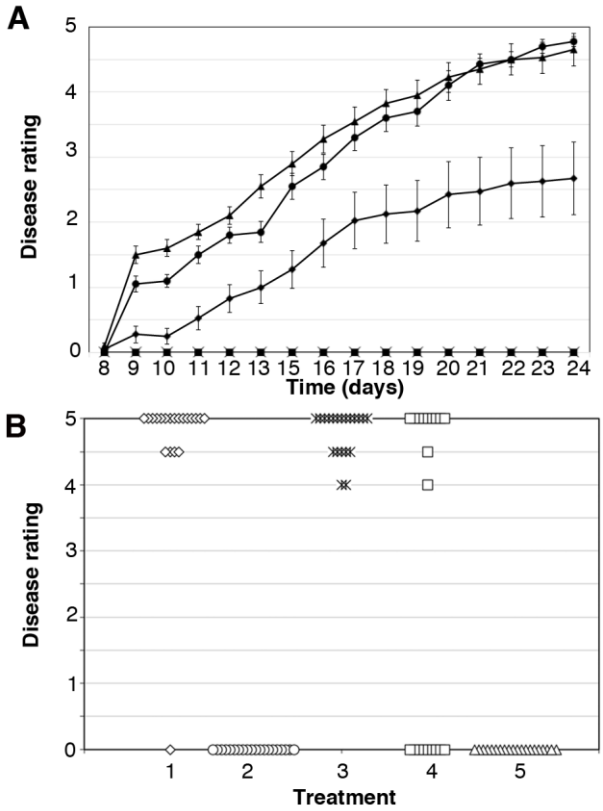


Fig. 4. ΔPD1311 inoculation to grape prior to TM1 suppressed PD development. A) Weekly mean disease ratings of vines inoculated with TM1-only (triangles), TM1 and ΔPD1311 simultaneously (circles), ΔPD1311 two weeks prior to TM1 (diamonds), ΔPD1311-only (squares) and buffer (x marks) respectively. Error 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 results are shown and discussed in the Summary section.

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	0cm[d]	30cm[d]	150cm[dc]m
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. This results are shown and discussed in the Summary section.

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 understand its insect transmissibility. Because ΔPD1311 has reduced aggregation and biofilm

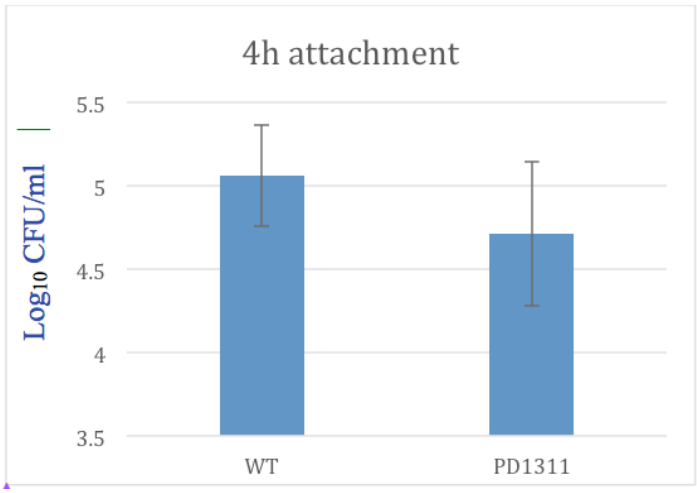


Fig. 5. The ΔPD1311 strain attached to leafhopper hind wings similarly to the wild-type strain. The attachment assay was performed as described previously (Baccari et al. 2014). The experiment was performed once with eight replicates included for each strain.

(**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 1c. Construction of deletion mutant of gene PD1311 was halted once it was noted results in 2016 were not consistent with previous years. This will be an important step once the status of current ΔPD1311 and WT strains are determined.

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.

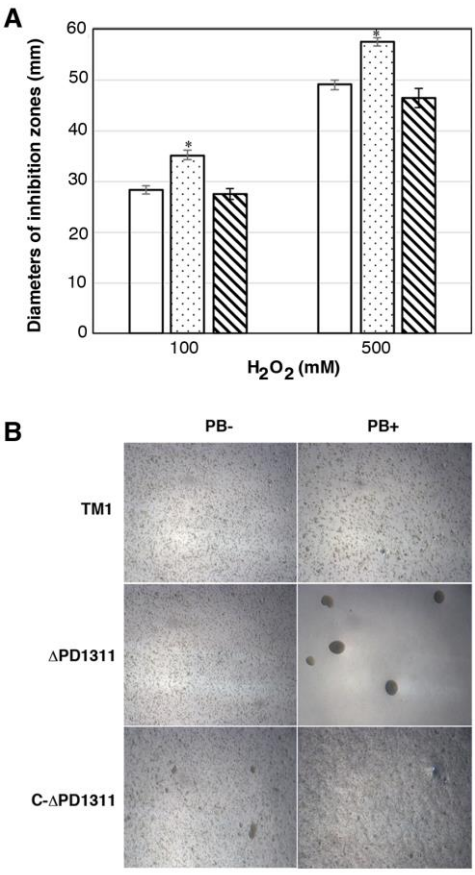


Fig. 6. Relative sensitivity of ΔPD1311 to H_2O_2 and polymixin B (PB). A) Mean diameters of inhibition zones of TM1 (empty bars), ΔPD1311 (dotted bars) and C-ΔPD1311 (dashed bars) exposed to 100 or 500 mM of H_2O_2 on PD2 agar plates. Error bars represent standard deviations. Three replicates were included 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.

VIII. SUMMARY OF ACCOMPLISHMENTS AND RESULTS FOR EACH OBJECTIVE.

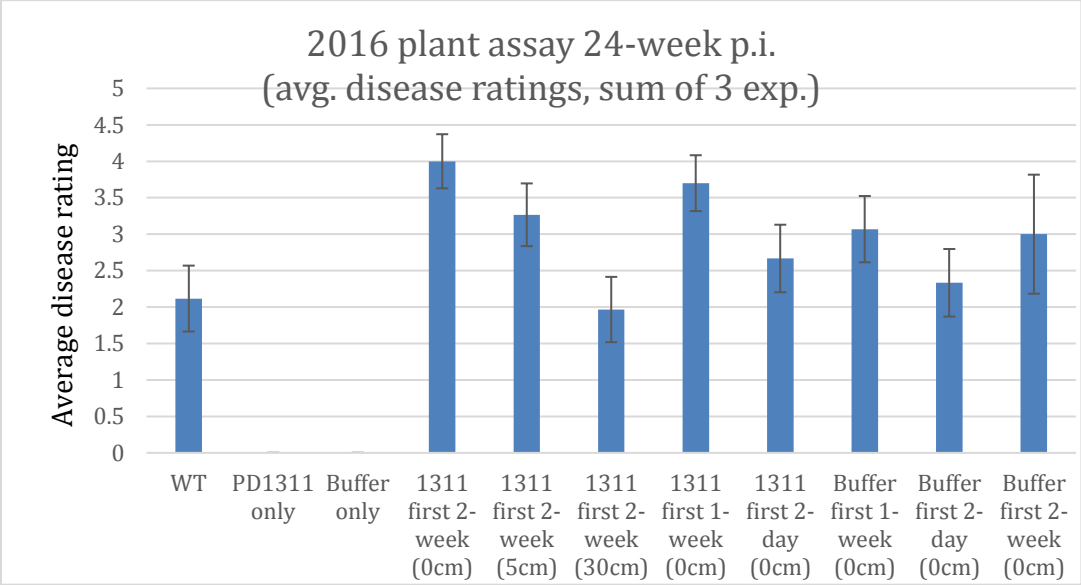
Concerning objective 1: Results from the 2016 inoculation experiments are shown in Table 3. Although ΔPD1311 was again confirmed as avirulent, we were unable to further verify the PD disease suppression of ΔPD1311. Plants inoculated with WT only developed about 40% infection which was much lower than in past years where infection level was close to 100%. The reason for this difference is unknown. We do not expect conditions in the greenhouse were involved as the grapevines were growing well and the internal climate was similar to past years. For treatments where ΔPD1311 was applied prior to WT the level of disease was frequently higher than the inoculation with WT alone. No disease developed when ΔPD1311 was applied alone. Differences were mostly observed when ΔPD1311 was applied two weeks prior to WT and was inoculated 30 cm above the WT inoculation point. For that treatment PD was less than when ΔPD1311 was inoculated at the point of WT inoculation of 5 cm above. We had not attempted the treatments of 5 cm or 30 cm previously. Disease suppression was observed over the past three years when WT was applied at the same inoculation site as ΔPD1311.

The significant differences that were observed in 2016 with regard to PD suppression by ΔPD1311 could also be due to a modification in the ΔPD1311 strain. Before going ahead with research on ΔPD1311 it will be essential to explore the possibility that the strain became altered in storage. Initially it would be important to test previously reported ΔPD1311 phenotypes including biofilm formation, aggregation and motility on synthetic media and sap agar. If it appears that the strain has changed from its original behavior we would check additional stocks or remake the mutant. This research was not funded in 2015 however we were able to conclude the experiments because of being granted a no-cost extension of funds that remained from 2015.

The experiments to test the effect of ΔPD1311 on PD development in overwintered plants was inconclusive because there was great variability in disease across all categories of plants. Some that showed disease during summer of 2015 did not develop disease in 2016 regardless of being treated with ΔPD1311 or not. Considering the results obtained from the experiments discussed above it would be necessary to repeat the experiments on overwintered plants once

factors that were involved in overall reduced disease and reduced inhibition of ΔPD1311 in the 2016 experiments are determined.

Figure 7. Effect of ΔPD1311 on PD development, 2016. Specific methods and conditions used for the different treatments are explained in the text above.



Preliminary data suggests that ΔPD1311 attaches to insect hindwings at an equal level as observed for wild-type cells. Therefore in nature ΔPD1311 could possibly be distributed by the vector.

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. It has also identified a key PD virulence factor, PD1311, that will be important in future research to understand the mechanism by which *X. fastidiosa* causes PD. Additional work on this essential putative enzyme is highly warranted.

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

Publications (Peer reviewed and Proceedings).

Hao L, Athinuwat D, Johnson K, Cursino L, Burr TJ, Mowery P. *Xylella fastidiosa pil-chp* operon is involved in regulating key structural genes of both type I and type IV pili. Vitis J Grapevine Res. Accepted.

Hao L, Johnson K, Cursino L, Mowery P, Burr TJ. Characterization of the *Xylella fastidiosa* PD1311 gene mutant and its suppression of Pierce's disease on grapevines. *Mol. Plant Path.* 2016 Jul 8 in press (doi: 10.1111/mpp.12428).

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* 11(8): e0160978.

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.

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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.

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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.

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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 identified phenotypes consistent with total loss of virulence. In addition to ΔPD1311 being avirulent it may also reduce the ability of wild-type *X. fastidiosa* to cause PD, however this phenomenon was not repeated in 2016. Further research is necessary to identify the function of PD1311 in relation to causing PD and also how it is able to inhibit the development of PD.

XI. LAY SUMMARY OF PROJECT ACCOMPLISHMENTS.

We discovered that deleting the *X. fastidiosa* Temecula 1 gene, PD1311, results in loss of ability to cause Pierce's Disease. For the first three years of research on this mutant we provided evidence that ΔPD1311 has potential as a biological control however for unknown reasons we were not able to repeat that phenomenon in 2016. This may be due to alterations in the pathogen or in the ΔPD1311 strain, or to other plant or environmental factors that we are not aware of. Given the agricultural importance of Pierce's Disease, it is critical to understand how ΔPD1311 exerts its effects and also the role PD1311 gene in causing PD. Options for managing Pierce's Disease are limited, which makes development of new control strategies critically important. The results further expand our understanding of Pierce's Disease and provide information in relation to controlling the disease.

XII. STATUS OF FUNDS.

All funds have been expended.

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.

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