

Interim Progress report for CDFA contract number 07-0175.

**BIOLOGICAL CONTROL OF PIERCE'S DISEASE OF GRAPEVINE WITH
BENIGN STRAIN OF *XYLELLA FASTIDIOSA***

Time period covered by the progress report: July 1, 2007 – July 1, 2012

PRINCIPAL INVESTIGATORS AND COOPERATORS

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List of objectives, and description of activities conducted to accomplish each objective:

Objective 1. To evaluate strain EB92-1 of *X. fastidiosa* subsp. *fastidiosa* for the biological control of Pierce's disease of grapevine in new plantings in the vineyard in California.

Southern California tests. For transplanting into the Bella Vista Vineyard in Temecula, 50 Orange Muscat were inoculated with the biocontrol strain (EB92-1) on June 26, and 50 were left untreated as controls. Fifty Cabernet Sauvignon/110R were treated and 50 were untreated controls. These plants were transported to Temecula and transplanted into plots in the Bella Vista Vineyard on July 21-22, 2008.

In September 2010, all the young plants in the Bella Vista vineyard appeared to have severe water and nutritional stress. Many plants died without ever having any visible PD symptoms, probably due to the lack of water and poor nutrition. It is difficult to discern whether the PD-like symptoms are due to water stress or whether water stress increases PD. In the Orange Muscat test, 35-40% of the vines had died after 2 years from something other than PD, probably lack of water. Twenty-two percent of the Cabernet Sauvignon also had died, probably from water stress. In both the Cabernet Sauvignon and Orange Muscat, many of the vines were severely stunted and barely reached the trellis wire after 3 seasons and more than 2 years. Therefore, the trials were abandoned.

To replace the lost tests in southern California, a replacement test was established in 2011 at UC Riverside. For transplanting into the UC Riverside vineyard, 100 Merlot/1103 plants and 100 Pinot Noir/1103 plants were obtained from Sunridge Nursery in March 2011 and maintained in UC Davis greenhouse. Fifty Merlot and 50 Pinot Noir were inoculated with EB92-1 in July 2011 and fifty plants of each cultivar were kept as untreated controls. These plants were maintained in the greenhouse for 6 weeks and then moved outside to harden them off. These plants were transported to Riverside in mid-October and transplanted into the plots at UCR. This planting time will reduce heat stress on the transplants and, hopefully, will give them the fall season to establish a strong root system. This should result in vigorous plants in the spring of 2012 for inoculation with the PD strain of *X. fastidiosa* by resident GWSS throughout the season.

Sonoma tests. For Preston Vineyards in Sonoma, 50 Barbera/110R and Viognier/110R from were inoculated with EB92-1 and 50 vines of each were left as untreated controls. These plants were transported to Sonoma and transplanted as replants for missing vines in a mature vineyard the last week of July, 2008. On August 26, 2009, these vines were mapped for symptoms. All of the Barbera vines appeared to be healthy with no PD symptoms. The block of Barbera did not appear to have any PD symptoms, even in the older vines and this test was abandoned because of the lack of disease.

In the Viognier test, there were a few vines that had minor yellow and/or necrotic leaf margins on the basal leaves in 2009, but there were no definitive symptoms. Minor PD symptoms began to develop in a very few vines in the Viognier test in 2010. However, there were fewer vines with PD symptoms in 2011 than in 2010. There were very few new symptomatic mature Viognier vines in the test area. After 3 years, the PD incidence in the test vines is very low (Table 1). Only 2 vines in the entire test had moderately severe symptoms and should normally be removed. Symptoms in the other symptomatic vines were very minor and these vines could recover.

Table 1. Biocontrol of PD in 2-year-old grapevines in Northern California vineyards on 9/6/2011.¹		
Cultivar	Untreated vines	EB92-1 treated vines
<i>Preston Vineyard, Sonoma</i>		
Voignier	6/48 (12%)	5/48 (10%)
<i>Beringer Vineyard, Napa</i>		
Chardonnay	0/42 (0%)	1/44 (2%)
Reisling	2/47 (4%)	0/44 (0%)
Total	8/137 (6%)	6/136 (4%)
¹ Disease incidence is given as number of PD symptomatic vines over total vines in treatment.		

Napa tests. For transplanting into the Beringer Vineyard in Napa, 50 Reisling/3309 and 50 Chardonnay/3309 were treated with EB92-1 on June 25, 2008 and 50 vines of each were left untreated as controls. The vines were transplanted as replants for missing vines in Beringer Vineyard in early April 2009. In the third season, there still is essentially no disease in either the Chardonnay or Reisling (Table 1). Only 1 Chardonnay vine and 2 Reisling vines were considered to have the beginning of PD symptoms, but these were still questionable.

Objective 2. To evaluate strain EB92-1 of *X. fastidiosa* for the protection of older established grapevines against Pierce's disease in California vineyards. Since PD is rapidly developing in the mature Chardonnay block at Beringer Vineyard in Napa, it was chosen for an evaluation of EB92-1 for the prevention of PD development in mature, producing grapevines. On September 8, 2010, forty vines were inoculated with EB92-1 and 40 vines were chosen as controls. The vines were inoculated with strain EB92-1 in the main trunk, approximately equidistant from the graft and the trellis wire. Vines were injected by boring a small hole into the trunk with an electric drill. Two ml of the bacterial suspension will be injected into each hole using a nail-injector syringe. In 2011, none of the mature vines, treated or untreated, had developed any PD symptoms.

Objective 3. To develop a PCR based assay that can quickly differentiate the PD biocontrol strain EB 92-1 from pathogenic, wild type *Xylella* strains. We will continue to evaluate the tandem repeat numbers (TRN) of additional PD Xf reference strains as well as samples that we collect from the biocontrol plots. For the field samples we will initially test approximately 6 to 8 individual colonies from any one sample to determine the degree of potential polymorphisms in colonies isolated from a symptomatic vine. DNA extraction is very straightforward as a single colony will be removed from a medium plate and suspended in 40ul of sdH₂O. The sample will be boiled for 3 minutes and 5ul of the supernatant DNA will be used as the PCR template. This method was used to prepare DNA template for the gel shown in Figure X. PCR conditions and primers to be used are described by Chen, et al., 2008. We will initially evaluate all PCR field samples using agarose gel electrophoresis to get a clear visual representation of the size and number of the PCR products in each sample.

We will also evaluate the efficacy of determining the size of the TRN PCR products using quantitative real-time PCR (QRT-PCR) following the methodology described by Bextine and Child, 2007. These researchers used QRT-PCR to differentiate gyrase B genes from 3 Xf strains that only differed in a few nucleotides. It is likely that TRNs that differ in size by many base pairs should be readily differentiated using this method. If successful, QRT-PCR has the advantage that samples will not have to be evaluated by gel electrophoresis. Determining the identity of an Xf strain in a vine would simply involve isolating Xf colonies on PD3, picking a representative number of colonies from the plate and directly analyzing their TRN number by QRT-PCR which generates unique temperature melting profiles for each size TRN.

EB 92-1 colonies have consistently produced the same size PCR fragment that differed in size from the PD wild type strains. Although our sample size has been comparatively small the initial results provide rationale for further evaluating this as a tool for differentiating EB- 92-1 from wild type Xf strains which will strengthen our ability to conclude whether EB 92-1 provides PD protection under California conditions.

Objective 4. To evaluate rapid, efficient methods of treatment with strain EB92-1 of *X. fastidiosa* for the biocontrol of PD in *V. vinifera* in the vineyard.

On May 29, 2007, Merlot/101-1 plants were injected with EB92-1 in the greenhouse. Treatments were (1) EB92-1 in scion only, (2) EB92-1 in rootstock only, (3) EB92-1 in both rootstock and scion, and (4) Nontreated. On June 21, vines were transplanted into the vineyard in 3 replications of 3 plants per treatment. On June 13, 2007, Chardonnay CL96/3309 were injected with EB92-1 in the greenhouse. Treatments were (1) scion only, (2) rootstock only, (3) rootstock plus scion, (4) nontreated, and (5) scion only after transplanting into vineyard (These injections were done on July 26). On July 3, vines were transplanted into the vineyard. The Chardonnay plants grew very poorly due to rootstock problems and were removed in the spring of 2010.

In 2009, PD began to occur in a few of the Merlot vines. There was no significant difference among treatments, but symptoms were very mild and often do not occur in the next season in plants treated with EB92-1. Two of the Merlot treatments had no symptoms. In 2010 and 2011, all three treatment methods resulted in less PD than in the untreated Merlot plants (Table 2).

Table 2. Effect of methods of treatment of grape plants with *Xylella fastidiosa* strain EB92-1 on biological control of Pierce's disease.

Treatment	% PD incidence in 6/25/11 in: ¹
	Merlot/101-14
Scion injection	17
Rootstock injection	22
Scion & Rootstock injection	11
Untreated	43

¹%PD is the number of plants with symptoms divided by total number of plants x 100.

Plants of Chardonnay/Salt Creek were obtained by grafting green cuttings from Chardonnay plants from the vineyard onto rooted cuttings of Salt Creek. The grafting was done between May and July in 2007. Grafted plants were transplanted into the vineyard on August 14, 2007. Treatments included (1) Cuttings from Chardonnay not infected with EB92-1 on Salt Creek, (2) Cuttings from EB92-1 inoculated Chardonnay on Salt Creek, and (3) Cuttings from Chardonnay not infected with EB92-1 on Salt Creek, but injected in the vineyard with EB92-1 on August 29.

Table 1. Transmission of biocontrol in scion from infected Chardonnay mother plant grafted onto Salt Creek rootstock.	
	% PD incidence: 5/25/12
Treatment	
Scion from clean Chardonnay	80
Scion from clean Chardonnay injected with EB92-1 in the field	18
Scion from EB92-1 Chardonnay mother plant	73

In 2012, the incidence of PD symptoms in the scion from clean Chardonnay was high (Table 1). As expected, field injection of these clean scion plants with EB92-1 reduced the incidence of PD from 80% to 18%. The scion wood from mother vines of Chardonnay infected with EB92-1 had almost as much PD the the uninfected scion wood had. Thus, transfer of the biological control from the mother plant through scion wood is highly questionable. Further development of the symptoms will continue to be observed. This evaluation of scion from treated mother vines is especially significant, because scion wood from infected mother vines would be a preferred treatment method over having to inject every vine by pin pricking.

Publications and presentations:

Hopkins, D. L. 2007. Biological control of Pierce's disease of grapevine with benign strains of *Xylella fastidiosa* subsp. *piercei*. IN: Proceedings of the Pierce's Disease Research Symposium, December 12-14, 2007, San Diego, CA, California Department of Food and Agriculture.

Poster at Pierce's Disease Research Symposium in San Diego, December 12-14, 2007

Hopkins, D.L. and Thompson, C.M. 2008. Biological control of Pierce's disease in the vineyard with a benign strain of *Xylella fastidiosa*. J. Plant Pathol. 90S:115.

Hopkins, D. L., B. Kirkpatrick, B. Hill, R. Smith, and D. Johnson 2008. Biological control of Pierce's disease of grapevine with benign strains of *Xylella fastidiosa* subsp. *piercei*. Pages 164-166, IN: Proceedings of the Pierce's Disease Research Symposium, December 15-17, 2008, San Diego, CA, California Department of Food and Agriculture.

Oral presentation at Pierce's Disease Research Symposium in San Diego, December 15-17, 2008.

Oral presentation at Texas PD Symposium on "Biological control of Pierce's disease with a benign strain of *Xylella fastidiosa*" at Fredericksburg Texas in April, 2009

Hopkins, D. L., B. Kirkpatrick, B. Hill, R. Smith, and D. Johnson 2009. Biological control of Pierce's disease of grapevine with benign strains of *Xylella fastidiosa*. Pages

129-132, IN: Proceedings of the Pierce's Disease Research Symposium, December 9-11, 2009, Sacramento, CA, California Department of Food and Agriculture.

Oral presentation on "Biological control of Pierce's disease with *Xylella fastidiosa* strain EB92-1 in Vineyards with Different Rates of Disease Development" at the Pierce's Disease Research Symposium in Sacramento, December 9-11, 2009.

Hopkins, D.L. 2010. Management strategies for Pierce's disease: An increasing threat to grape production in the southern US. *Phytopathology* 100(6):S201.

Hopkins, D. L. 2010. Biological control with *Xylella fastidiosa* strain EB92-1 for the prevention of Pierce's disease development in mature, producing grapevines. *Phytopathology* 100(6):S52.

Oral presentation at Pierce's Disease Research Symposium in San Diego, December 15-17, 2010.

Hopkins, D. L., B. Kirkpatrick, B. Hill, R. Smith, and D. Johnson 2010. Biological control of Pierce's disease of grapevine with benign strains of *Xylella fastidiosa*. Pages 187-190, IN: Proceedings of the Pierce's Disease Research Symposium, December 15-17, 2010, San Diego, CA, California Department of Food and Agriculture.

Oral presentation on "Biological control of Pierce's disease of grapevine with eb92-1, a benign strain of *Xylella fastidiosa*" at the Biological Control of Grapevine Diseases in Toulouse, France on May 26-27, 2011.

Hopkins, D. L., B. Kirkpatrick, B. Hill, R. Smith, and D. Johnson 2011. Biological control of Pierce's disease of grapevine with benign strains of *Xylella fastidiosa*. Pages 128-131, IN: Proceedings of the Pierce's Disease Research Symposium, December 13-15, 2011, Sacramento, CA, California Department of Food and Agriculture.

Relevance to Solving the PD Problem in CA:

The successful completion of the proposed research could lead to an effective control of Pierce's disease that is environmentally friendly. The strains utilized in this study are naturally occurring and are not genetically modified in any way. Thus, we would avoid the concerns associated with introducing genetically modified organisms or plants. This should lead to faster implementation than could be attained with genetically engineered plants or biocontrol organisms. A commercial company has licensed the patent and is preparing to register this strain with the EPA for commercial control of PD and a commercial formulation of EB92-1 should be on the market in 3-4 years (2014/2015) to control PD of grapevine in California and other areas where PD is a problem.

Layperson Summary of Project Accomplishments:

Trial plantings of Orange Muscat and Cabernet Sauvignon were established in Bella Vista Vineyard in Temecula for the biocontrol of PD with a benign strain of *Xylella*. Many of the vines were stunted or have died from something other than PD, probably water stress. These trials in Temecula had to be abandoned. In 2011, the lost Temecula trials were replaced with trials on Merlot and Pinot Noir in the UC Riverside Vineyard. The vines were transplanted in October. In Preston Vineyards in Sonoma, a trial on Barbera was abandoned because there was no PD. There is a low level of PD in the remaining Viognier trial. In Beringer Vineyards in Napa, there is a very low level of PD in the Chardonnay and Reisling trials. A trial to evaluate the effectiveness of the biocontrol strain in protecting mature, producing grapevines against infection with PD was established in Beringer Vineyard in 2010. Mature Chardonnay vines were inoculated with biocontrol strain EB92-1 by boring a small hole into the trunk with an electric drill and injecting 2 ml of bacterial suspension into the hole using a nail-injector syringe. After one season, none of the mature vines, treated or untreated, had developed any PD symptoms, further illustrating the lack of disease pressure this year. Due to the lack of PD or other cultural problems, none of the tests have produced sufficient data to tell us whether or not the biocontrol strain is effective in California. Tests are also underway in Florida to determine the most efficient and effective way to apply the biocontrol strain. The biocontrol seems equally effective when applied to the rootstock or the scion. In attempts to stop the development of PD in mature vineyards, drilling the main trunk and injecting the biocontrol strain with a syringe was more effective than pin-pricking injections of current season growth.

Status of funds:

There are sufficient funds to get us through June 30 2012, when the project is scheduled to end. However, a small amount of additional funding is needed to monitor the field trials for 2 more years.

Intellectual property issues:

None, UF owns patent on strain, EB92-1

Literature cited:

Hopkins, D. L. 2005. Biological control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. Plant Dis. 89:1348-1352.