July 2010 Progress report for CDFA contract number 07-0175.

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

Time period covered by the progress report: July 1, 2007 – July 1, 2010

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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* which has provided effective biocontrol of PD in previous greenhouse and vineyard tests in Florida for possible commercial application for the biological control of Pierce's disease of grapevine in the vineyard in California.

All plants for the vineyard tests were planted in April 2008 in greenhouses at UC Davis. The cultivars were Orange Muscat (propagated by the grower, Imre Cziraki, and starting budbreak when planted April 6), Cabernet Sauvignon/110R (dormant rooted vines from Vintage Nursery, planted April 30), Reisling/3309 (dormant rooted vines from Vintage Nursery, planted April 30), Chardonnay/3309 (dormant rooted vines from Vintage Nursery, planted April 30), Barbera/110R (dormant rooted vines from Sunridge Nursery, planted April 30, and Viognier/110R (growing potted vines from Vintage, planted April 30.

The biocontrol strain, EB92-1 was recovered from storage in glycerol at -70 C. Five and 6-day cultures of second transfer of the bacterium from storage on PD3 solid medium were hand-carried by Don Hopkins on a flight to California. For biocontrol treatment of the grape plants, a slightly cloudy solution of EB92-1, approximately 0.25 OD at 600 nm (10^7 – 10^8 CFU/ml) was prepared in 75 ml of SCP buffer (disodium succinate, 1.0 g/L;

trisodium citrate, 1.0 g/L; K_2HPO_4, 1.5 g/L; KH_2PO_4, 1.0 g/L; pH 7.0) in Bruce Kirkpatrick's laboratory at UC Davis. A pin pricking technique was used to inoculate the biocontrol into the xylem vessels of the treated grapevine. A drop (0.02 ml) of the biocontrol suspension was placed onto each of two lower internodes of the plants. The stem was pierced 3-5 times through the drop with a syringe needle. The inoculum was pulled into the plant by the negative pressure of the pierced xylem vessels. Approximately 5×10^5 to 5×10^6 bacteria were inoculated into each node.

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 late fall 2008, PD-like symptoms were observed in most of the vines at Bella Vista, treated or untreated (Observation by Barry Hill). However, it was very hot and dry in 2008 and some of these symptoms may have been due to the weather. In the summer of 2009, PD symptoms were still extensive in the Bella Vista Vineyard, but were observed in only about half of the vines that had symptoms in 2008. Differences in the incidence of PD between the treated and untreated vines were not significant (Table 1). Symptoms did appear to be more severe in the untreated Cabernet Sauvignon vines than in the EB92-1 treated vines. The Orange Muscat planting was interspersed with mature vines that were nearly 100% infected with Pierce's disease. This entire planting, except our experimental vines probably will be removed this year. There have been similar experiments in Georgia and Florida where leafhoppers were abundant and 40% of the vines developed PD in a single year. Under this situation, EB92-1 did not influence PD incidence during that year, but provided good control in the 2nd and 3rd years. The effectiveness of the biocontrol has appeared to increase after the first year in the vineyard. The next two years will indicate whether the biocontrol can be effective under the severe disease pressure in this Temecula vineyard.

Table 1. Effect of EB92-1 on PD incidence in new grape plantings transplanted on July 21-22, 2008 into Bella Vista Vineyard in Temecula.

	8/25/09	
Treatment	**% PD[1]**	**Rating[2]**
	Cabernet Sauvignon	
Untreated	35	1.9
EB92-1 treated	40	1.4
	Orange Muscat	
Untreated	50	2.3
EB92-1 treated	44	2.2

[1]Percentage of total vines that have PD symptoms.

[2]Disease rating was an average per symptomatic vine on a scale of: 1 = any symptom of PD, such as marginal necrosis (MN) on a basal leaf or two; 2 = moderate marginal necrosis, more than 5 %; and 3 = severe symptoms.

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 the last week of July, 2008. On August 26, 2009, these vines were mapped for symptoms. Most of the Viognier vines had been pruned back to a two bud spur last winter. There were no definite symptoms on August 26. There were a few vines that had minor yellow and/or necrotic leaf margins on the basal leaves of the 2009 growth. Some of these were sampled for the presence of the PD pathogen or the biocontrol strain EB92-1. All of the Barbera vines appeared to be healthy with no PD symptoms. The Viognier block has significant PD incidence in the mature vines and these test vines should begin to develop PD symptoms in 2010. The block of Barbera did not appear to have any PD symptoms, even in the older vines. The disease pressure appears to be very low in this Barbera block.

For transplanting into the Beringer Vineyard in Napa, 50 Reisling/3309 and 50 Chardonnay/3309 were treated with EB92-1 on June 25 and 50 vines of each were left untreated as controls. The vines were transplanted in Beringer Vineyard in early April 2009. On August 26, these vines had not started to develop PD symptoms. Many of these vines were exhibiting drought stress.

Objective 2. To compare different methods of treatment with strain EB92-1 of *X. fastidiosa* subsp. *fastidiosa* for the biocontrol of PD in *V. vinifera* in the vineyard. Cuttings of the cultivars Chardonnay and Chambourcin (French/American hybrid) in the UF Mid-Florida REC vineyard were taken both from vines that are colonized by biocontrol strain EB92-1 and vines not colonized by *X. fastidiosa*. Rooted cuttings of these vines were potted in the greenhouse and 12 of the cuttings from untreated vines were injected with strain EB92-1. Two weeks later all plants were inoculated with

Table 2. Comparison of treatment method with EB92-1 on control of Pierce's disease in the greenhouse.

	Pierce's disease rating after 8 wks:[1,2]	
Source of EB92-1 treatment	**Chardonnay**	**Chambourcin**
Untreated rooted cuttings	2.9 b	4.1 b
Rooted cutting from field EB92-1, biocontrol plant	3.0 b	2.8 a
Injected EB92-1 untreated rooted cuttings	2.2 a	2.6 a

[1]Plants were rated on a 0 - 5 scale with 0 = no symptoms and 5 = a dead plant. Ratings were averaged for treatments.

[2]Mean separation in columns by Duncan's New Multiple Range Test, 5% level.

pathogenic PD strains and observed weekly for symptoms. In both cultivars, plants injected with strain EB92-1 in the greenhouse had significantly lower PD rating than the untreated plants (Table 2). There did not seem to be any effect of taking the cuttings from an infected vine in the vineyard with Chardonnay, but PD was significantly less severe in the plants derived from cuttings of biocontrol vines of the cultivar Chambourcin than in plants derived from untreated vines.

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 (Table 3). 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 the spring of 2010, all three treatment methods resulted in less PD than in the untreated Merlot plants.

Table 3. Effect of methods of treatment of grape plants with *Xylella fastidiosa* strain EB92-1 on biological control of Pierce's disease.

	Merlot/101-14	
Treatment	**Sep 2009**	**May 2010**
Scion injection	0	13
Rootstock injection	0	11
Scion & Rootstock injection	25	0
Untreated	11	33

In a third experiment, Chardonnay cuttings from the MREC vineyard were grafted onto Salt Creek rootstock rooted cutting from the vineyard. The grafted plants were transplanted into the vineyard on August 14. The treatments included 1) Chardonnay cuttings from mature vines that had been treated 3 years ago with EB92-1 on Salt Creek, 2) Chardonnay cuttings from mature nontreated vines on Salt Creek, and 3) Chardonnay cuttings from mature nontreated vines on Salt Creek, with the scion injected with EB92-1 in the vineyard on August 29.

In 2009, the incidence of PD symptoms in the scion from clean Chardonnay was high (Fig. 1). As expected, field injection of these clean scion plants with EB92-1 reduced the incidence of PD from 70% to 9%. Plants developed using scion wood from mother vines of Chardonnay infected with EB92-1 had slightly less PD than plants developed with uninfected scion wood. This indicates that there could be some transfer of the biological

control from the mother plant through scion wood. Further development of the symptoms will 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.

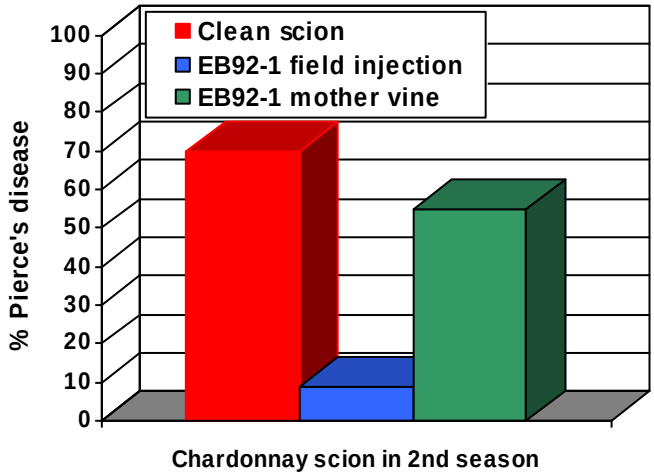

Fig. 1. Transmission of biocontrol in scion from infected Chardonnay mother plant grafted onto Salt Creek rootstock.

While strain EB92-1 has been shown to be effective in preventing PD in new grape plantings, there are mature vineyards that are rapidly being destroyed by PD. To evaluate control of PD in older vines, mature vines were treated either by two pin-pricking injections of a drop of EB92-1 into a current year shoot on each of the major arms (branches) or by by drilling a hole and injecting 0.5 – 1.0 ml of EB92-1 with a syringe. Drill and syringe injection of the main trunk in these mature vineyards with chronic PD was more effective than pin-pricking in reducing new cases of PD in American hybrid grapevines during the first year after treatment (Fig. 2).

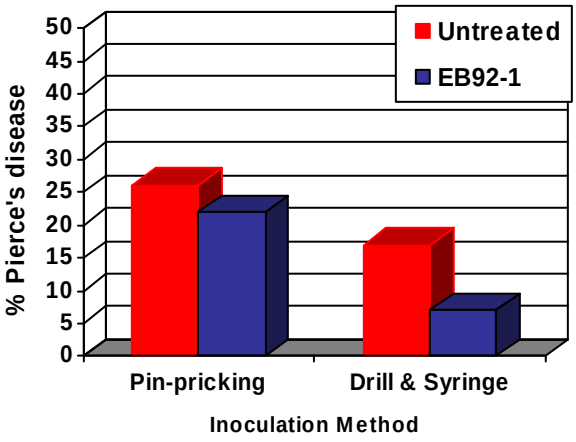

Fig. 2. Effect of strain EB92-1 on PD in mature vines treated by pin-pricking compared with drill and syringe in 2009 trials.

Intellectual Property Issues:

None, UF owns patent on strain, EB92-1

References, publications, reports, and presentations:

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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.

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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.

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 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 2-3 years (2012/2013) to control PD of grapevine in California and other areas where PD is a problem.