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

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Reporting Period: The results reported here are from work conducted July 2007 to September 2008.

ABSTRACT
This project includes the evaluation of the biological control of Pierce's disease (PD) in California with a strain of *Xylella fastidiosa*, EB92-1, which has provided effective control of PD in previous greenhouse and vineyard tests in Florida. On June 26, 2008 in greenhouses at UC Davis, fifty plants of Orange Muscat, Cabernet Sauvignon, Reisling, Chardonnay, Barbera, and Viognier were inoculated with strain EB92-1 and fifty plants of each were left untreated as controls. The Orange Muscat and Cabernet Sauvignon vines were transplanted into the Bella Vista Vineyard in Temecula on July 21-22. Barbera and Viognier were transplanted into Preston Vineyards in the Sonoma Valley during the last week of July and Reisling and Chardonnay were transplanted into Beringer Vineyard in Napa Valley in October. The development of PD in these trials will be monitored for two-five years. In Florida, different methods of obtaining young plants colonized with strain EB92-1 were evaluated for the effectiveness in biological control of PD. In the greenhouse, rooted Chambourcin cuttings from vines in the UF research vineyard that had been treated with EB92-1 had fewer PD symptoms than rooted cuttings from control vines; however, Chardonnay rooted cuttings from treated vines had similar PD symptoms as from the untreated. In the vineyard at Mid-Florida REC, scion, rootstock, and scion plus rootstock treatments with EB92-1 all had significantly lower incidences of PD than the untreated vines of Merlot and there were no significant differences among these treatments.

INTRODUCTION
Pierce's disease (PD) of grapevine is a chronic problem for the California grape industry and has become more of a threat to the industry with the introduction of the glassy-winged sharpshooter. PD is especially damaging in the southeastern USA where it is endemic and is the primary factor limiting the development of a grape industry based on the high-quality European grapes (*Vitis vinifera* L.). The only feasible control for Pierce's disease is resistance. Through 10 years of research on the biological control of Pierce's disease of grapevine in Florida by cross protection with weakly virulent strains of *Xf*, we demonstrated that this also is a potential means of controlling this disease. In a vineyard study, *Xf* strain EB92-1, benign to grapevine, provided excellent control of PD in *V. vinifera* cv. Cabernet Sauvignon for four years in the vineyard in central Florida (Hopkins, 2005). Strain EB92-1 was introduced into the vines only once at the beginning of the 4-year trial and was still controlling the disease at the end; whereas, all unprotected vines were dead. These treated vines were still healthy and producing fruit in 2008, 11 years after treatment. The overall goal of this project is to develop a biological control system for Pierce's disease (PD) of grapevine that would allow the production of *V. vinifera* in California and other areas where PD and the glassy-winged sharpshooter (GWSS) are endemic.

OBJECTIVES
1. To evaluate strain EB92-1 of *Xf* 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.
2. To compare different methods of treatment with strain EB92-1 of *Xf* for the biocontrol of PD in *V. vinifera* in the vineyard.

RESULTS
Establishment of field trials of strain EB92-1 for biological control of PD in vineyards in California
It took from July to December 2007 to obtain the USDA Permits to test the biocontrol strain in California. The field plot locations are in the Bella Vista Vineyard in Temecula, CA, in the Beringer vineyard in the Napa Valley, and in Preston Vineyards in the Sonoma Valley.

All plants for the vineyard tests were planted in April 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 six-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 three-five 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 x 10^5 to 5 x 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..

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. 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 October.

Comparison of treatment methods with strain EB92-1 for biocontrol of PD

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 *Xf*. 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 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 1**). 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. This may mean that the biocontrol strain is not consistently carried over into propagated plants. Recent experiments have indicated that the 0.25 OD inoculum of pathogen can overcome the biocontrol strain in some cases; therefore, this experiment is being repeated with lower pathogen inoculum levels.

Table 1. Comparison of treatment method with EB92-1 on control of PD in the greenhouse.

	PD 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.

Experiments to evaluate different methods of treatment with EB92-1 were established in the Mid-Florida REC vineyard during the summer, 2007. Four treatments were applied to the cultivar Merlot/101-14 (dormant rooted vines from Vintage Nursery planted in pots in mid-April) on May 29 and the plants were transplanted into the vineyard on June 21. The treatments were 1) injection of EB92-1 into the new growth of the scion only, 2) injection of EB92-1 into the rootstock only, 3) injection of EB92-1 into both the rootstock and scion, and 4) nontreated. Five treatments were applied to the cultivar Chardonnay CL96/330914 (dormant rooted vines from Vintage Nursery planted in pots in mid-April) on June 13 for the three greenhouse treatments and on July 26 for the scion field injection.. The plants were transplanted into the vineyard on July 3. The treatments were 1) injection of EB92-1 into the scion only in the greenhouse, 2) injection of EB92-1 into the rootstock only in the greenhouse, 3) injection of EB92-1 into both the rootstock and scion in the greenhouse, 4) nontreated, and 5) injection of EB92-1 into the scion only in the vineyard. 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 three 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.

One year after these trials were established, PD incidence was still low and there were not any significant differences between treatment methods and the untreated in Chardonnay/3309 (**Table 2**). However, on October 7, 2008, scion, rootstock, and scion plus rootstock treatments with EB92-1 all had significantly lower incidences of PD than the untreated vines of Merlot. This early, preliminary data indicates that it may not be critical whether the strain EB92-1 is injected into xylem of rootstock, scion, or both. Disease development in these trials in years two and three is most important to any conclusions on the most effective treatment methods. In the first year, there were no significant differences among the Chardonnay/Salt Creek treatments. This evaluation of cuttings from treated vines is especially significant, because rooting cuttings from infected mother vines would be a preferred treatment method over having to inject every vine by the pin pricking method.

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

	% PD incidence in:[1]	
Treatment	Merlot/101-14	Chardonnay/3309
Scion injection	0 a	0
Rootstock injection	0 a	0
Scion & Rootstock injection	11 a	11
Scion field injection	NT	11
Untreated	33 b	0

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

CONCLUSIONS

There are no results or conclusions for the California field trials, since the vines treated with strain EB92-1 were not established in vineyards until mid-summer to fall of this year. Preliminary results in Florida indicated that rooted cuttings from EB92-1 mother vines did not consistently have reduced incidence of PD in greenhouse tests. This method of using the biocontrol strain may not be feasible, but field tests are underway to evaluate this treatment method, because rooting cuttings from infected mother vines would be a preferred treatment method over having to inject every vine by the pin pricking method. As symptoms of PD began to develop in Merlot in Florida trials, the strain EB92-1 appeared to reduce PD incidence whether it was applied to rootstock or scion.

REFERENCES CITED

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.