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

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ABSTRACT
In the Bella Vista Vineyard in Temecula, loss of plants to extreme water stress and nutritional problems have forced abandonment of the trial on Orange Muscat and, probably, will also eliminate the Cabernet Sauvignon trial. In the Orange Muscat test, 35-40% of the vines had died after two years from something other than Pierce's disease (PD), probably water stress. In Preston Vineyards in Sonoma, EB92-1 was reducing the incidence of PD in Viognier when compared to untreated vines in this first year of characteristic PD symptom development in the trial. The test on Barbera has been discontinued due to lack of PD. With adequate irrigation, both the Chardonnay and Reisling test vines in the Beringer Vineyard in Napa were vigorous and grew well in this second growing season. PD began to develop in the test vines of both varieties and trends were for reduced PD in the EB92-1 treatments compared to the untreated. In comparisons of methods of treatment with EB92-1 in three-year-old Merlot vines, EB92-1 was controlling PD equally well after injection into the rootstock, scion, or rootstock and scion. Vines developed using scion wood from mother vines of Chardonnay infected with EB92-1 had less PD than vines developed with uninfected scion wood or even from clean plants injected with EB92-1. Development of plants with scion wood from infected mother vines could eliminate the need to inject every vine by pin pricking.

LAYPERSON SUMMARY
In trial plantings of Orange Muscat and Cabernet Sauvignon in Bella Vista Vineyard in Temecula, almost half of the Orange Muscat, treated or untreated vines have died from something other than Pierce's disease (PD), probably water stress. Many of the surviving vines were severely stunted and barely reached the trellis wire after more than two years and three growing seasons. This makes it impossible to obtain good data so the trial has been abandoned. Approximately a fourth of the Cabernet Sauvignon also have died and this test will be abandoned unless plant survival and vigor improve. Since there was no PD in the Barbera in Preston Vineyards in Sonoma, no data can be obtained from this test either. The other three tests are going well. In Preston Vineyards in Sonoma, EB92-1 was controlling PD in Viognier when compared to untreated vines. In Beringer Vineyard in Napa, PD began to develop in the tests on Chardonnay and Reisling and trends were for reduced PD in the EB92-1 treated compared to the untreated. In both the Sonoma and Napa trials, symptoms are just beginning to develop so it is early to draw definitive conclusions on the control trends. We are evaluating the use of mother vines infected with the biocontrol strain EB92-1 as propagation material for scion wood. In 2010, vines developed using scion wood from mother vines of Chardonnay infected with EB92-1 had less PD than vines developed with uninfected scion wood. This indicates that there could be transfer of the biological control from the mother plant through scion wood. Development of plants with scion wood from infected mother vines could eliminate the need to inject every vine by pin pricking.

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 (GWSS) (Hopkins and Purcell, 2002). 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 PD is resistance. The results of our 10 years of research on the biological control of PD of grapevine by cross protection with weakly virulent strains of *X. fastidiosa (Xf)* have demonstrated that this is a potential means of controlling this disease (Hopkins, 2005). We have identified at least one strain that was able to control PD in *V. vinifera* for 14 years in Central Florida. We are testing this strain in commercial vineyards on a limited basis and, if these tests are successful, the strain will be ready for commercial use. The overall goal of this project is to develop a biological control system for PD of grapevine that would allow the production of *V. vinifera* in California and other areas where PD and the GWSS are endemic.

In previous research with the biocontrol strain, the bacteria were injected into the grapevines after they were transplanted into the vineyard. This is a labor-intensive procedure. Three methods in order of increasing desirability are vineyard injection, nursery injection, and propagating wood from mother vines that are infected with the biocontrol strain. We are currently

evaluating injection of the biocontrol strains into the vines in the nursery, prior to transplanting into the vineyard. The use of scion or rootstock propagating wood from mother vines that are already infected with the biocontrol strain would make this technology less labor-intensive, less costly, and more consistent. It would eliminate any variability in the relative effectiveness of injections into different plants.

OBJECTIVES

1. To evaluate strain EB92-1 of *Xf* for the biological control of PD of grapevine in new plantings in the vineyard in California.
2. To evaluate strain EB92-1 of *Xf* for the protection of older established grapevines against PD in California vineyards.
3. To develop a PCR based assay that can quickly differentiate the PD biocontrol strain EB 92-1 from pathogenic, wild type *Xf* strains.
4. To evaluate rapid, efficient methods of treatment with strain EB92-1 of *Xf* for the biocontrol of PD in *V. vinifera* in the vineyard.

RESULTS AND DISCUSSION

Field trials evaluating strain EB92-1 for biological control of PD in vineyards in California

All plants for the vineyard tests in the Bella Vista Vineyard in Temecula, in the Beringer vineyard in the Napa Valley, and in Preston Vineyards in the Sonoma Valley were planted in April, 2008 in greenhouses at UC Davis. 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.

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. All of the vines, treated and untreated, were under severe water stress and this may have caused some of the PD-like symptoms. Differences in the incidence of leaf scorch between the treated and untreated vines were not significant. The Orange Muscat planting was interspersed with mature vines that were nearly 100% infected with PD. This entire planting, except our experimental vines were removed during the winter of 2009, leaving only our young plants scattered in the vacant vineyard.

In September 2010, all the young plants in the Bella Vista vineyard appeared to have severe water and nutritional stress. PD-like symptoms were extensive in the plants that were still alive, treated and untreated. Many plants died without ever having any 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 two 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 three seasons and more than two years. Therefore, the Orange Muscat test is definitely lost. The Cabernet Sauvignon test will probably have to be abandoned also, but better irrigation and fertilization could salvage it. We will re-evaluate it in 2011.

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. The disease pressure appears to be very low in this Barbera block. In September 2010, there were still no symptoms in the Barbera block, either in the new test vines or the older vines. This test will not be evaluated in 2011, 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. The Viognier block has significant PD incidence in the mature vines and these new test vines began to develop PD symptoms in 2010 (**Table 1**). Symptoms were not very severe, but there were more symptomatic vines in the untreated vines than in the EB92-1 vines. This trend indicated that EB92-1 was reducing the incidence of PD in the Viognier. With the amount of symptoms in the mature Viognier vines, PD should continue to develop in the young test vines.

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. On August 26, 2009, these vines had not started to develop PD symptoms. Many of the vines were exhibiting drought stress.

Table 1. Biocontrol of PD in two-year-old grapevines in Northern California vineyards, 9/8/10.				
	EB92-1 treated vines:		**Untreated vines:**	
Cultivar	**#PD vines/total**	**Disease rating[1]**	**#PD vines/total**	**Disease rating[1]**
Beringer Vineyard, Napa				
Chardonnay	3/45 (7%)	0.1	4/48 (8%)	0.1
Reisling	4/47 (9%)	0.1	6/51 (12%)	0.1
Preston Vineyard, Sonoma				
Viognier	8/48 (17%)	0.2	13/48 (27%)	0.3
UTotalU	15/140 (11%)	0.1	23/147 (16%)	0.2
[1]Disease rating was an average per vine on a scale of: 0 = no symptoms; 1 = any symptom of PD, such as marginal necrosis (MN) on a basal leaf; 2 = definite, moderate symptoms on <50% of vine; 3 = severe symptoms on >50% of vine; 4 = dead plant.				

With better irrigation, both the Chardonnay and Reisling test vines in the Beringer Vineyard were vigorous and grew well in this second growing season. PD began to develop in the test vines of both varieties (**Table 1**). The trends were for less PD in the EB92-1 treatments and more PD in Reisling than Chardonnay. However, these are very early results as the plants are only in their second season and less than two years old. With extensive PD in the mature Chardonnay and Reisling vines, disease should continue to develop in the young test vines.

Field trial evaluating EB92-1 for the protection of older established grapevines against PD 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. Randomly, forty vines were inoculated with EB92-1 and 40 vines were chosen as controls. On September 8, 2010, 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.

Comparison of treatment methods with strain EB92-1 for biocontrol of PD
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 three plants per treatment. In 2009, PD began to occur in these vines, but there were no obvious differences between treatments.

In 2010, the EB92-1 treated vines had less PD than the untreated (**Table 2**). PD biocontrol was obtained whether EB92-1 was injected into the scion, the rootstock, or both. The treatments appeared to be equally effective.

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

	% PD incidence in August 2010 in:[1]
Treatment	**Merlot/101-14**
Scion injection	13
Rootstock injection	11
Scion & Rootstock injection	14
Scion field injection	-
Untreated	38

[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. In the first year, there were no significant differences among the Chardonnay/Salt Creek treatments.

In 2009, plants developed by injecting clean scion with EB92-1 in the field or by using scion wood from mother vines of Chardonnay infected with EB92-1 had less PD than plants developed with uninfected scion wood. In 2010, injection with EB92-1 and the use of scion wood from mother vines infected with EB92-1 had less PD than untreated vines (**Table 3**). Surprisingly, there was less PD in plants developed from scion wood from EB92-1 infected mother plants than in plants that

were injected directly with EB92-1. While it may be too early to draw conclusions, this indicates that there could be 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 could be an efficient treatment method that does not require a lot of additional hand labor over normal production practices.

Table 3. Transmission of biocontrol in scion from infected Chardonnay mother plant grafted onto Salt Creek rootstock.

Treatment	**% PD incidence in August 2010:**
Scion from clean Chardonnay	40
Scion from clean Chardonnay injected with EB92-1 in the field	27
Scion from EB92-1 Chardonnay mother plant	9

CONCLUSIONS

In three-year-old Merlot vines in Apopka, FL, EB92-1 was controlling PD equally well after injection into the rootstock, scion, or rootstock and scion. Vines developed using scion wood from mother vines of Chardonnay infected with EB92-1 had less PD than vines developed with uninfected scion wood or from uninfected scion wood injected with EB92-1. Development of plants with scion wood from infected mother vines could eliminate the need to inject every vine by pin pricking.

In the Bella Vista Vineyard in Temecula, loss of plants to extreme water stress and nutritional problems have forced abandonment of the test on Orange Muscat and, probably, will also destroy the Cabernet Sauvignon test. If vigor can be restored to the plants, it may be possible to salvage the Cabernet Sauvignon test. This will be determined in 2011. In Preston Vineyards in Sonoma, the Barbera test was discontinued because there was no PD, but the Viognier block was doing very well. In this first year of clear symptom development, EB92-1 was reducing the incidence of PD in Viognier. In this second growing season for tests in Beringer Vineyard in Napa, both the Chardonnay and Reisling tests were growing very well and PD was developing in both tests. There were no significant differences yet, but there was a trend toward less PD in the EB92-1 treated vines. Of the six tests of biocontrol of PD in new plantings established in 2008-2009, two tests will probably have to be discontinued due to plant death from water stress and nutritional problems and one test discontinued due to the lack of PD. Early in the other three tests, EB92-1 appears to be reducing PD incidence. The successful completion of the biocontrol tests in Temecula, Sonoma, and Napa would lead to an effective control of PD that is environmentally friendly. This project should yield results within the next two years and if the control is successful, there should be a biological control for PD available for commercial use in vineyards in California.

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