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 from extreme water stress and nutritional problems has forced abandonment of the trials on Orange Muscat and Cabernet Sauvignon. To evaluate *Xylella fastidiosa* strain EB92-1 for the biocontrol of Pierce's disease (PD) in Southern California, a new test site was established at the UC Riverside vineyard in October 2011. Fifty treated and 50 untreated Merlot and Pinot Noir vines were transplanted in mid-October. After three years in Preston Vineyards in Sonoma, there was no PD in the Barbera trial, which has been abandoned, and a very low level of PD in the Viognier trial. There were fewer vines with PD in 2011 than in 2010. In the third season in the Beringer Vineyard in Napa, there still is essentially no disease in either the Chardonnay or Reisling. Only one Chardonnay and two Reisling vines out of a total of 177 vines were considered to have the beginning of PD symptoms. In September 2010, forty mature Chardonnay vines in the Beringer Vineyard were inoculated with 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 with a nail-injector syringe. In 2011, 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 loss of trials in Temecula and the lack of PD development in trials in Sonoma and Napa, no useful information on the biological control of PD with EB92-1 in California has been obtained after three years of trials. In comparisons of methods of treatment with EB92-1 in four-year-old Merlot vines in Florida, EB92-1 was controlling PD equally well after injection into the rootstock, scion, or rootstock and scion. Control of PD in older, mature vines was evaluated by comparing pin-pricking injections of a drop of EB92-1 into a current year shoot on each of the major arms and injections by drilling a hole in the main trunk 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.

LAYPERSON SUMMARY
Trial plantings of Orange Muscat and Cabernet Sauvignon were established in Bella Vista Vineyard in Temecula for the biocontrol of Pierce's disease (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 are being 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.

INTRODUCTION
Pierce's disease (PD) of grapevine is an endemic, chronic problem in the southeastern USA where it is the primary factor limiting the development of a grape industry based on the high-quality European grapes (*Vitis vinifera* L.) (Hopkins and Purcell, 2002). PD is also endemic in California and has become more of a threat to the California grape industry with the introduction of the glassy-winged sharpshooter. While vector control has been effective for PD control in some situations,

the only long-term, feasible control for PD has been resistance. Almost 20 years of research on the biological control of Pierce's disease of grapevine by cross protection with weakly virulent strains of *Xylella fastidiosa (Xf)* has demonstrated that this is a potential means of controlling this disease (Hopkins, 2005). One strain of *Xf* that was able to control PD in *V. vinifera* for 14 years in Central Florida has been identified. We are testing this strain in commercial vineyards in several states and, if these tests are successful, the strain will be ready for commercial use. In most trials with the biocontrol strain, the bacteria were injected into the grapevines either in the greenhouse or in the vineyard after transplanting. This is a labor-intensive procedure. Treatment methods that would make the technology less labor-intensive, less costly, and more consistent are being evaluated. The overall goal of this project is to develop a biological control system for PD of grapevine that would control the disease in California and other areas where PD and the glassy-winged sharpshooter (GWSS) are endemic.

OBJECTIVES

1. To evaluate strain EB92-1 of *Xf* for the biological control of Pierce's disease 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 Pierce's disease 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 *Xylella* 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

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 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, with no significant differences in the incidence of PD between the treated and untreated vines. 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 PD.

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 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 two years. Therefore, the trials were abandoned.

To replace the lost tests in southern California, a replacement test is being 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 six 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 *Xf* 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 three years, the PD incidence in the test vines is very low (**Table 1**). Only two 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**
Preston Vineyard, Sonoma		
Voignier	6/48 (12%)	5/48 (10%)
Beringer Vineyard, Napa		
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 one Chardonnay vine and two Reisling vines were considered to have the beginning of PD symptoms, but these were still questionable.

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.

In 2011, none of the mature vines, treated or untreated, had developed any PD symptoms, further illustrating the lack of disease pressure this year.

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 three replications of three plants per treatment.

In 2009, PD began to occur in a few of the Merlot vines. Symptoms have continued to progress in the untreated, with 43% of the vines having some symptoms (mostly minor) in 2011 (**Table 2**). All three treatments with EB92-1 were reducing symptoms. There were very few new PD infections in 2011 when compared with 2010.

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

	Merlot/101-14	
Treatment	**Aug 2010**	**June 2011**
Scion injection	13	22
Rootstock injection	11	13
Scion & Rootstock injection	14	17
Untreated	38	43

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 pin-pricking injections of a drop of EB92-1 into a current year shoot on each of the major arms (branches) or by drilling a hole in the main trunk 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 (**Figure 1**).

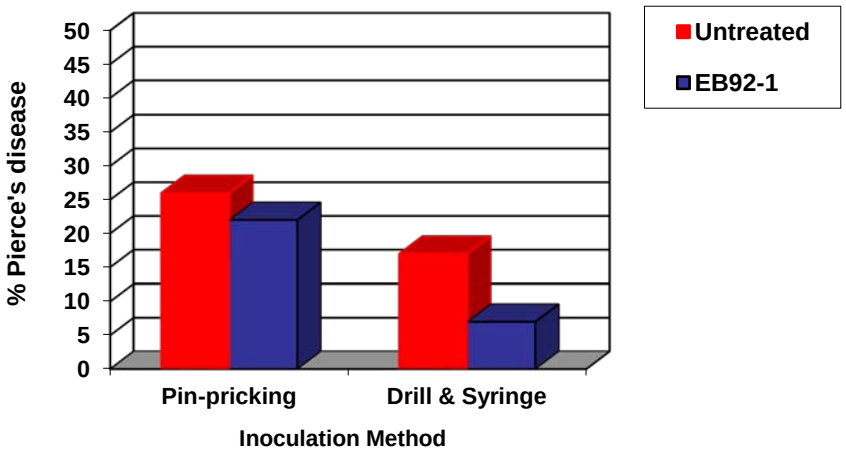

Figure 1. Effect of strain EB92-1 on PD in mature vines treated by pin-pricking compared with drill and syringe in 2009 trials**.**

CONCLUSIONS

In four-year-old Merlot vines in Florida, a trial was established to determine the most efficient and effective way to apply the biocontrol strain. EB92-1 was controlling PD equally well after injection into the rootstock, scion, or rootstock and scion. In attempts to stop the development of PD in mature vineyards, drilling the main trunk and injecting the biocontrol with a syringe was more effective than pin-pricking current season growth. Due to the loss of trials in Temecula and the lack of PD development in trials in Sonoma and Napa, no definitive information on the biological control of PD with EB92-1 in California has been obtained after three years of trials. To replace the lost tests in southern California, a test is being established in 2011 at UC Riverside. With the PD pressure in southern California, this test will yield conclusive results over the next 2-3 years. Hopefully, the Sonoma and Napa tests will develop enough PD to give us an evaluation in those areas over the same time period. If the control is successful in the current trials, along with success in other states, this project could yield results within the next 2-3 years that would provide a commercial biological control for PD for vineyards in California.

REFERENCES

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.

Hopkins, D. L. and Purcell, A.H. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis. 86:1056-1066.

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