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

Principal Investigator:
Donald L. Hopkins
Mid-Florida REC
University of Florida
Apopka, FL 32703
dhop@ufl.edu

Cooperators:
Bruce Kirkpatrick
Dept. of Plant Pathology
University of California
Davis, CA 95616
bckirkpatrick@ucdavis.edu

Barry L. Hill
Calif. Dept. Food & Agric.
Sacramento, CA 95814
bhill@cdfa.ca.gov

Rhonda Smith
Farm Advisor
Sonoma County
Santa Rosa, CA 95403

Drew Johnson
Beringer Vineyards
Napa, CA 94559

Reporting Period: The results reported here are from work conducted July 2007 through September 2009.

ABSTRACT
Fifty Orange Muscat and Cabernet Sauvignon/110R treated with *Xylella fastidiosa (Xf)* strain EB92-1, along with 50 untreated controls of each, were transplanted into plots in the Bella Vista Vineyard in Temecula on July 21-22, 2008. In August 2009, Pierce's disease (PD) symptoms were extensive in the Bella Vista Vineyard, with 35-50% of the vines having symptoms in this first full season in the vineyard. Differences in the incidence of PD between the treated and untreated vines were not significant. Symptoms were generally mild and were less severe in the EB92-1 treated Cabernet Sauvignon vines than in the untreated vines. There were no definitive PD symptoms in the Beringer Vineyard in Napa and only a few possible PD symptoms in the Preston Vineyard in Sonoma. In comparisons of methods of treatment with EB92-1 in 2009, symptoms began to develop in Chardonnay and Merlot that had been injected in different tissues with the biocontrol strain. There were no significant differences in PD among vines injected in the scion, rootstock, or both. In 2009, the incidence of PD symptoms in the UF vineyard with scion from clean Chardonnay was high. Field injection of these clean scion plants with EB92-1 reduced the incidence of PD from 70% to 9%. The scion wood from Chardonnay mother vines infected with EB92-1 had slightly less PD than the uninfected scion wood. This indicates that there could be some transfer of the biological control from the mother plant through scion wood, which would be a preferred treatment method over having to inject every vine by pin pricking.

LAYPERSON SUMMARY
In a test planting of Orange Muscat and Cabernet Sauvignon in Bella Vista Vineyard in Temecula, almost 50% of the vines had developed Pierce's disease (PD) symptoms by August 2009, only 13 months after transplanting. The incidence in the vines treated with EB92-1 was very similar to the vines not treated. Under these kinds of severe PD pressure and high populations of leafhopper vectors in tests in Florida and Georgia, it is often the second year after planting before a beneficial effect from the biocontrol is observed. PD development over the next two years should determine whether the biocontrol strain EB92-1 will be effective under these extreme conditions. In the biocontrol tests in Preston vineyard in Sonoma and Beringer Vineyard in Napa, symptoms that are PD-like have only occurred in a vine or two. There should be enough disease to begin to evaluate the biocontrol in these vineyards next year. Currently, treatment with the biocontrol strain involves needle injection of every grapevine with a suspension of the bacteria. This is a laborious and time-consuming job. We are evaluating the use of mother vines infected with the biocontrol strain EB92-1 as propagation material for scion wood and/or rooted cuttings. If the biocontrol strain can be transmitted through scion wood, it would eliminate the inoculation step. In 2009, vines developed using scion wood from mother vines of Chardonnay infected with EB92-1 had slightly less PD than the uninfected scion wood had after two years in the vineyard in Florida. This indicates that there could be some transfer of the biological control from the mother plant through scion wood. Further observation of these plants will be made next year.

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 (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 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 13 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 glassy-winged sharpshooter (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 which has provided effective biocontrol of PD in previous greenhouse and vineyard tests in Florida for possible commercial application for the biological control of PD 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 AND DISCUSSION

Establishment of field trials of 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. 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 PD. 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 reduce PD incidence during that year, but slowed the spread in the second and third 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.**

Treatment	% PD[1]	8/25/09 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, 2008 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.

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 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 MREC vineyard.

In 2009, PD began to occur in a number of these vines, especially in the Chardonnay test (**Table 2**). There was no significant difference among treatments, but symptoms were very mild and often do not develop any further in plants treated with EB92-1. There was less PD in the Merlot plants, with two treatments having no symptoms.

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

	% PD incidence in September 2009 in:[1]	
Treatment	**Merlot/101-14**	**Chardonnay/3309**
Scion injection	0	29
Rootstock injection	0	43
Scion & Rootstock injection	25	33
Scion field injection	-	29
Untreated	11	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, the incidence of PD symptoms in the scion from clean Chardonnay was high (**Table 3**). 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.

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

	% PD incidence in September 2009:
Treatment	
Scion from clean Chardonnay	70
Scion from clean Chardonnay injected with EB92-1 in the field	9
Scion from EB92-1 Chardonnay mother plant	55

CONCLUSIONS

The biocontrol strain did not reduce the incidence of PD in the Bella Vista Vineyard in Temecula. Leafhopper vectors are not controlled in this vineyard and PD incidence is extremely high in the vineyard. Plants treated with EB92-1 did appear to have milder symptoms and than the untreated plants. Symptoms are just beginning to develop in the tests in Sonoma and Napa. Vines developed using scion wood from mother vines of Chardonnay infected with EB92-1 had slightly less PD than vines developed with uninfected scion wood after two years in the vineyard in Florida. Development of plants with scion

wood from infected mother vines would be a preferred treatment method over having to inject every vine by pin pricking. 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.

REFERENCES 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.

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.

FUNDING AGENCIES

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