BIOLOGICAL CONTROL TRIALS WITH EB92-1 IN TEXAS

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LAYPERSON SUMMARY

There are no direct control recommendations for the management of Pierce's disease (PD) of grapes caused by *Xylella fastidiosa (Xf)*. As a result, losses in productivity to this recalcitrant bacterium are heavy in Texas with sometimes disastrous consequences for winegrape growers. Disease control still depends largely on suppressing insect vector populations, eliminating competing weeds and supplemental hosts, and the use of less desirable, resistant grape cultivars. A new biological control option has been successfully tested in other states for protecting high risk, susceptible vines with a "benign" strain of the pathogen. This strain, designated EB92-1, was originally isolated from an elderberry plant and shown to cause only mild, or no symptoms when inoculated into grape. Several experimental approaches previously have been used to demonstrate the protective properties of the benign strain. These approaches include both greenhouse and vineyard tests where plants were inoculated with EB92-1 prior to artificial inoculation with the pathogen. The use of a benign strain of a pathogen to protect plants from a more virulent strain of the same pathogen has been demonstrated in numerous other plant diseases. There are unique reasons why such a biocontrol agent might be realistic for the PD problem on grapes. Should the use of EB92-1 successfully protect grapevines in Texas from colonization by *Xf*, this will be the first and only direct control method designed to increase vine productivity growing in high risk PD regions.

INTRODUCTION

This project is designed to test a potential biological control agent for Pierce's disease (PD) of grapes, caused by *Xylella fastidiosa (Xf)* subsp. *Piercei*. Biological control agents of plant diseases come in many forms (Agrios 2005). Their success not only depends on properties of the pathogen and the host, but also on the economics involved in production of the affected crop. PD of grapes has the attributes for which biological control would be a particularly attractive approach for management (Hopkins 1989, Purcell and Hopkins 1996, Raju and Wells 1986). The crop is very high in value, is intensively managed and the pathogen threatens grapevines over a wide range of the area where they are grown. Should a biological control be proven effective, there will be a huge demand for its use. Also, *Xf* has a complex population structure in which there is a wide variety of strain specificity occurring throughout a very large host range. The cross infectivity among these hosts makes it possible for the bacterium to successfully colonize different hosts while eliciting a variety of responses, some of which may be minimal or non-existent.

Xf is a native, endemic pathogen in Texas. PD is a limiting factor for growth of *Vitis vinifera* varieties in many of the winegrape regions in the state. Current recommendations for PD control can be expensive and inconsistent. As a result, growers face a great deal of anxiety over sustained production in existing vineyards, as well as a lack of confidence in selecting varieties for replanting and establishing new vineyards. These circumstances present the ideal environment in which to test a potential biocontrol agent to reduce losses from PD.

One approach for biocontrol of a plant disease is the introduction of a unique strain of the relevant pathogen that can mitigate the properties of the virulent, problematic strain of the same pathogen. Tests have shown that a strain of *Xf* from elderberry can be inoculated into grape and reduce the ability of the virulent, native grape strain to cause disease (Hopkins 2005, Hopkins et al. 2007). This strain, designated EB92-1 has been applied to grapevines and successfully reduces disease severity when the vines were subsequently challenged by a virulent strain of *Xf* (Hopkins 2005).
This project would be a new line of investigation under the Texas Pierce's Disease Research and Education Program. The use of EB92-1 is already being tested in the California Program (Hopkins et al. 2007). This project would compliment the California research and add valuable information to the use of this potential biocontrol under conditions found in Texas. The proposed project would be one of the few projects in the Texas Program aimed at directly controlling the pathogen in the vine. Although a management program for PD exists for Texas growers (Kamas et al., 2004), losses continue to mount throughout all winegrape regions in the State. If successful, it would represent a valuable addition to our ability to prolong vine productivity and relieve the current losses being sustained by Texas grape producers.

The following Objectives are proposed to thoroughly test the use of EB92-1 under a variety of conditions as a potential biocontrol for PD of grapes in Texas. Each is needed to investigate promising conditions for which the potential biological control agent might be applied.

OBJECTIVES

1. Treat grapevines in greenhouses with EB92-1 prior to inoculation with a native virulent grape strain of *Xf* subsp. *fastidiosa.*
2. Treat grapevine cuttings with EB92-1 prior to planting into vineyards at high risk to *Xf.*
3. Treat grapevines in infected vineyards with EB92-1 to test for preventive and therapeutic properties of the potential biocontrol agent.

RESULTS AND DISCUSSION

Objective 1 Greenhouse tests. The elderberry strain EB92-1 was inoculated into potted grapevines either alone or four weeks prior to inoculation with a fresh grapevine-strain of the pathogen. There were 10 vines of four varieties (Merlot, Viognier, Cabernet Sauvignon, and Blanc du Bois) in each of the treatments. A proven needle-inoculation technique was used to directly introduce the bacterium (adjusted for concentration of 10^5 to 10^6 CFU/ml) into the vascular system of the vine (Hopkins and Adlerz 1988, Hopkins 2005). Two additional treatments consisted of the grapevine strain alone and a buffer inoculated control. The greenhouse trials were initiated in June, 2009 and monitored weekly for symptom development and survival for two growing seasons. Typical symptoms were quantified and verified utilizing ELISA (enzyme linked immunosorbent assay) and/or QRT-CPR (quantitative real time – polymerase chain reaction). Symptoms, or a disease rating scale of 1 - 6 was used, where 1 = healthy, 2 = 5-40% scorch, 3 = 50 - 90% scorch, 4 = 100% scorch with no dieback, 5 = 100% scorch with dieback, and 6 = dead.

At the end of the first growing season, some trends in the responses of vines to the treatments were evident (**Figure 1**). Merlot and Viogner exhibited the greatest level of symptom development when inoculated only with the grapevine strain of the pathogen. The Cabernet sauvignon and Blanc du Bois did not react negatively in any of the treatments. The EB92-1 inoculations resulted in some symptom development, but also appeared to suppress symptoms in the combination treatment relative to the grape strain inoculations. At the end of the second growing season (**Figure 1, B**.), the trends were very similar to the first. One notable difference was the development of symptoms in the Cabernet sauvignon in the grape strain inoculations, but again, the combined treatment appeared to suppress symptoms in that variety.

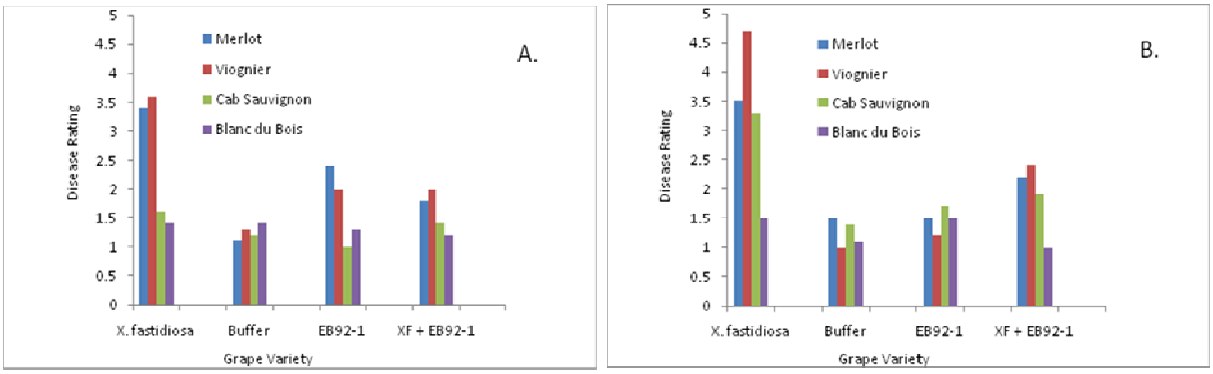

Figure 1. Average disease ratings (A. September, 2009 and B. September, 2010) for grapevines injected with 4 treatments consisting of a grape strain of *Xf*, a benign strain (EB92-1), both, or a buffer control. Vines were treated in June 2009.

Objective 2. Treated cuttings. Cuttings of the same four varieties used in Objective 1were grown in greenhouses prior to treatment and transplanting to vineyards in June – July, 2009. Ten vines of each variety were treated by inoculating the lower stem in the same manner described in Objective 1. Treatments consisted of a bacterial suspension of *Xf* strain EB92-1 (n = 10), a buffer "inoculation" (n = 10), and 10 untreated vines. Four weeks after inoculation, the vines were planted in Palacios Vineyard, a production vineyard near Brenham, TX. This location is considered to be a high risk vineyard for natural infection by native *Xf* subsp. *piercei* and has been the focus of numerous PD research projects. The vines were monitored monthly for development of typical PD symptoms, rated, and analyzed as described for Objective 1.

There were no symptoms of natural infection during the first growing season in any of the treatments, nor have any symptoms developed thus far during the 2010. The levels of PD in Palacios vineyard have been much lower during the 2010 growing season, probably due to frequent rainfall, massive roguing efforts, weed control and vector management. This plot

will continue to be monitored and will be sampled for analyses with ELISA and QRT-PCR to assess the level of infections in the vines.

Objective 3. Mature vine treatments in vineyards. This objective was accomplished by inoculating mature, high risk vines in two vineyards with the potential biocontrol *Xf* strain EB92-1. The first was Palacios Vineyard where Objective 2 was completed. Four varieties were treated, including Merlot, Shiraz, Cabernet sauvignon, and Blanc du Bois. Vines for treatment were selected in blocks according to records of vine health and current observations regarding status of infection by *Xf*. There were two treatments consisting of an injection with EB92-1 (n = 5), a buffer injection (n = 5), and 5 vines were left untreated in the block. The two treatments and untreated checks were repeated 4 times for each variety, bringing the total number of vines for each variety in the experiment to 60. A set (n = 15 vines) of the three groups were arranged in sequence in 3 – 4 adjacent rows. Bacterial suspensions were prepared as in the first two objectives. However, 4 – 6 ml of bacterial suspension or buffer was injected into the stems of the vines with a 50 ml syringe. Vines were be monitored in the same manner as in Objective 2. Mean disease severity ratings for the treatments will be compared separately.

As in the case of Objective 2, the low level of disease development in Palacios vineyard during the 2010 growing season has obscured any obvious potential treatment effects to date. None of the Blanc du Bois vines show any signs of scorching. Only two Merlot vines have developed symptoms, each in the untreated set of vines. In the Cabernet sauvignon, 2 untreated vines and 3 vines treated with EB92-1 have developed symptoms. One vine injected with buffer has developed symptoms in the Shiraz.

The treatment of mature vines was repeated at the Fredericksburg Experimental Vineyard in Fredericksburg, TX. The treatments were similar to those at Palacios but with more varieties. An analysis of the Fredericksburg plots was underway at the time of submission of this report.

CONCLUSIONS

These experiments are testing the ability of the elderberry strain of *Xf* (EB92-1) to protect grapevines from the effects of the more aggressive grape strain of the pathogen. Of the three experiments, the greenhouse inoculations gave the most convincing case for suppression of disease development in vines inoculated with a preventive dose of the biocontrol strain prior to inoculating with the virulent strain. The effect was seen best in two varieties, Merlot and Viognier, but further analyses are needed to determine the significance of these results. These are probably the most susceptible of the four varieties. Blanc du Bois is considered to be tolerant to *Xf*, and this was again confirmed in the greenhouse inoculations. Although Cabernet sauvignon exhibited some resistance in the first year of treatment with the *Xf* grape strain, the vines may be succumbing in the second year. This experiment will be maintained and analyzed for at least one more year to determine if the biocontrol is lasting, and whether there is a varietal influence on the ability of EB92-1 to protect vines. However, these preliminary results are encouraging.

Results of the field trials will also need more time to become sufficient for proper analyses. During the interim, we will be sampling vines for ELISA and QRT-PCR to determine the presence of the pathogen and assist in evaluating their health in the absence of symptoms. A few of the vines inoculated with EB92-1 have developed classic PD symptoms in both greenhouse studies and field trials. This was also noted to occur in previous studies, but was shown to have no lasting detrimental impact on vine health. At this point it should not be considered problematic for the potential use of EB92-1 in prolonging the productivity of grapevines at high risk to infection by the grape strain of *Xf*.

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