EVALUATION OF BACTERICIDES AND MODES OF DELIVERY FOR MANAGING PIERCE'S DISEASE

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ABSTRACT

For the past four years we have been evaluating plant micronutrients and inducers of systemic acquired resistance as prophylactic agents to protect grapevines against infection by *Xylella fastidiosa (Xf)* or as therapeutic agents to cure Pierce's disease (PD) affected grapevines. To date**,** none of the plant micronutrients containing zinc, copper or manganese or compounds that induce systemic acquired resistance in plants such as Actiguard^{R}, Messenger^{R}, or Resist^{R} showed efficacy when used as prophylactic agents to prevent infection of *Xf* in healthy grapevines, both in field trials and, for some materials, greenhouse evaluations of materials applied to potted grapevines that were then exposed to *Xf*-infectious sharpshooter vectors.

Evaluation of plant micronutrients and two antibiotics as therapeutic agents for curing, or at least causing a remission of symptoms in PD-affected grapevines, were more encouraging. Many of the PD-affected Merlot grapevines that were injected with zinc or streptomycin in Fall, 1999 and Spring, 2000 remained free of PD symptoms 3 years following treatment, whereas nearly all of the untreated control vines were dead. However, inspection and evaluation performed in October 2003 revealed that some of the vines that were symptomless in 2002 have now developed some early symptoms of PD. These symptoms may be the result of residual *Xf* populations that were not killed by the bactericide treatment finally multiplying to levels where they induced disease symptoms or these early infections may be the result of recent inoculation of treated vines by infectious-PD vectors. Field evaluation of a citrus terpene-based bactericide in Fall, 2002 did not reduce *Xf* populations in treated vines and all of these treated vines had progressed symptoms of PD in Fall, 2003.

INTRODUCTION

Bactericides such as fixed coppers, zinc, and antibiotics have been used with some degree of success in preventing some plant diseases. However, except for some phloem-limited pathogens such as phytoplasmas, all of the bacterial diseases in which bactericides have been of some value involve bacterial pathogens that reside on the surface of plants. Pierce's disease is caused by a xylem-limited bacterium, *Xylella fastidiosa* (*Xf)* which, to date, has not been successfully managed with bactericides. Although *Xf* is quite sensitive to a number of common bactericides that have been used on agricultural crops (Jones and Kirkpatrick, 2000) the major impediment to treating grapevines prophylactically to prevent infection by *Xf* or therapeutically to cure PD-affected vines has been the inability to introduce bactericides into grapevine xylem. If methods could be developed to effectively deliver prophylactic or therapeutic bactericides into grapevines, this could provide a comparatively straightforward solution to a very complex disease problem. Although genetic resistance to PD is probably the ultimate solution the ability to produce PD-resistant, commercially acceptable, multiple varieties and clones of *Vitis vinifera* will be a challenge. The development of a successful bactericide solution, particularly a prophylactic approach that would prevent infection of healthy vines, could be used as a viable interim solution to PD until more desirable approaches are developed to manage or prevent PD.

OBJECTIVES

1. Determine the efficacy of plant micronutrients and antibiotics as potential therapeutic bactericides for eliminating *Xylella fastidiosa* (*Xf*) in grapevines.
2. Determine the efficacy of plant micronutrients and systemic acquired resistance inducers to provide prophylactic protection against *Xf* infection of grapevines.

RESULTS AND CONCLUSIONS

Prophylactic Trials

Previously established prophylactic trials in Napa, Temecula, and Santa Cruz received three consecutive treatment applications this spring as opposed to the two treatments applied in previous years. Numbers of treated and control vines that subsequently developed Pierce's disease (PD) were still low in most of the trials; subsequently, these trials have not provided useful information at this time. However, two of the prophylactic trials, a White Riesling and a Merlot vineyard in Napa

experienced higher levels of infection and provided some insight into the potential efficacy of some treatments, especially those that appeared to offer little or no protection against *Xf* infection (Table 1).

Therapeutic Trials

Disease rating data from two Merlot trials in Napa was evaluated for efficacy following the final disease rating taken on September 11, 2002. Data for treatments that provided statistically significant benefit for suppressing PD symptoms, compared to the untreated controls for two Merlot trials, one vineyard rated 3 years after treatment and the second vineyard rated 2 years after treatment, is presented in Tables 2a, 2b. The results suggest that the most effective method for delivering the bactericides was the drill-through agar injection method and that zinc and streptomycin were two materials that provided significant positive results in both vineyards. The 2003 results were similar to rating data obtained in 2002. The 2001 results lead to the establishment of a third therapeutic trial in Fall, 2001 which tested the five best therapeutic injection treatments and one new treatment, phosphorous acid, in one of the previously used Merlot vineyards. It is too early to differentiate the beneficial effects of severe pruning, which was used in combination with all of the bactericide treatments, from the any effect of the applied bactericide in this plot. In Fall, 2002 two new therapeutic trials were established, one trial in Napa and one in Sonoma. A Sauvignon Blanc vineyard with mild to severe symptomatic vines was chosen in Napa, and four agarose injection treatments including copper sulfate, zinc sulfate, streptomycin, and phosphorous acid were applied. Each treatment was replicated on 15 vines and 15 untreated control vines were marked. The same treatment regime was repeated in the Sonoma Chardonnay vineyard with equally symptomatic vines. All bactericide treatments were applied in October of 2002.

A new root drench treatment was also tested in these two vineyards. The root drench product containing citrus terpenes was applied to 90 diseased vines in Napa at a rate of 1 gallon per vine with a second application one week after the first application. In addition, 20 diseased vines received a single 2-gallon dose with no additional applications. Untreated diseased vines were established as controls. A similar design was established in Sonoma using only the 1X rate. Petioles from treated vines were processed and evaluated for viable *Xf* cells. All of the treated vines still contained viable *Xf* cells at populations that were similar to untreated, diseased vines. This result was confirmed by a colleague in Georgia. The treated vines also had delayed shoot growth that was typical of a PD-infected vine and in October 2003 all of the terpene treated vines in Napa and Sonoma had typical symptoms of PD. These results indicate that the terpene had no beneficial effect in treated *Xf*-infected vines in Northern California.

Table 1. Percentage of vines in each disease-rating category for two prophylactic trials located in a White Riesling and a Merlot vineyard located in Napa, CC. Data reflects total amount of disease that occurred in 2001 through 2003. Disease Ratings = 0) healthy, 1) 1 or a few PD canes, 2) symptomatic canes throughout canopy, 3) cordon dieback, 4) dead vine.

			Percentage of healthy and PD vines as per 10/20/2003				
Vineyard	**Treatment**	**Reps**	**0**	**1**	**2**	**3**	**4**
White Riesling	ZincAA	20	60	10	5		25
	MnAA	20	65	5			30
	Rezist 2% + Stabilizer 2%	20	45				55
	Actigard	20	60				40
	Messenger	20	65	10			25
	Manganese Carbonate (4X)	20	70	5	5		20
	Zinc 50 (4X)	20	70				30
	Zn sulfate –soil applied	20	60				40
	Untreated Control	**20**	**65**	**10**			**25**
Merlot	ZnAA	20	60	15			25
	MnAA	20	75	5			20
	Rezist 2% + Stabilizer 2%	20	60	15	10		15
	Actigard 1X	20	80	10			10
	Messenger	20	65	5			30
	Manganese Carbonate (4X)	20	60	25			15
	Zinc 50 (4X)	20	65	5	10		20
	Zn sulfate – soil applied	20	50	5	5		40
	Untreated Control	20	70	10	5		15

Table 2a. Results of Cumulative Logit Model for disease rating in Merlot Vineyard A THREE years after initial treatment. Only treatments that were positively significant at 90% level are presented.

Treatment	Application Method	p	Parameter Estimate	Std. Error	Odds Ratio
Manganese	Injection	0.01	2.28	1.00	9.77
Streptomycin	Injection	0.004	2.86	1.08	17.54
Zinc sulfate	Injection	0.002	2.91	1.07	18.27
Zn/Mn	Foliar spray	0.01	2.88	1.3	17.81
Zn sulfate	Foliar spray	0.02	2.71	1.26	15.04

Table 2b. Results of Cumulative Logit Model for disease rating in Merlot Vineyard B TWO years after initial treatment. Only treatments that were positively significant at 90% level are presented.

Treatment	Application Method	p	Parameter Estimate	Std. Error	Odds Ratio
Zinc sulfate	Injection	0.06	1.45	0.79	4.26
Streptomycin	Injection	0.01	1.25	0.78	3.5
Tetracycline	Injection	0.01	1.25	0.78	3.5
Zn sulfate	Soil applied	0.05	2.71	1.26	15.04

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