Protection of Grapevine Pruning Wounds from Eutypa Dieback

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ABSTRACT

MOLLER, W. J., and A. N. KASIMATIS. 1980. Protection of grapevine pruning wounds from Eutypa dieback. Plant Disease 64:278-280.

After inoculation with ascospores, grapevine pruning wounds were highly susceptible to the vascular pathogen *Eutypa armeniacae* for several days after late winter pruning; susceptibility then rapidly decreased. Wounds made on 1-yr-old wood of *Vitis vinifera* vines in California were more resistant than those made on older tissue, and wounds that were hand-painted immediately with the systemic fungicide benomyl at 10,000 ppm (a.i.) were significantly protected from infection. Thiabendazole at either 10,000 or 20,000 ppm was less effective.

Additional key words: *Cytosporina*, deadarm

The ascomycetous fungus *Eutypa armeniacae* Hansf. & Carter (impf. *Cytosporina*) was recently recognized as an important parasite of grapes; it incites xylem necrosis near pruning wounds and causes death of shoots and arms (1,2). Although the disease is widespread, its etiology has been confused, and the "deadarm" symptoms have frequently been attributed to other pathogens, notably *Phomopsis viticola* Sacc. No data are available concerning susceptibility and protection of grapevine wounds, but results of previous control studies on *E. armeniacae* as a pruning wound pathogen in apricot orchards can be expected to be relevant for vineyards. High levels of benzimidazole fungicides (> 2%) hand-painted onto large apricot tree wounds afforded excellent protection for 6 wk or longer, by which time wounds were largely immune to infection (3,4).

Research was supported in part by the Wine Institute, San Francisco, CA.

This article reports research involving pesticides and does not contain recommendations for their use. All uses of pesticides must be registered by appropriate state and/or federal agencies before they can be recommended.

Accepted for publication 15 October 1979.

Eutypa dieback is a concern in California vineyards, especially where large pruning wounds are made to alter the training system or change the grape cultivar. This article reports experiments on grapevines during 1977-1979 to determine the effects of wound age and wood age on infection by *E. armeniacae* and the efficacy of two fungicide paints.

MATERIALS AND METHODS

Grapevines for inoculation. Forty-eight 10-yr-old grapevines (*Vitis vinifera* 'Grenache') growing in a vineyard at the University of California, Davis, were used for inoculations. The natural infection level in this area is low, and the vines had no visibly weakened or dead arms. The vines had been trained to a bilateral cordon with five to eight arms on each of two cordon branches. At the normal pruning time in February, arms were shortened to expose wounds of 2–3 cm diameter. Unless otherwise indicated, all experimental wounds were made on 2-yr-old wood and tagged with plastic surveyors' tape immediately below the pruning stub.

Inoculum. The inoculum was prepared by immersing small pieces of grape perithecial stromata in water for 1 hr, then using floral clay to suspend the pieces from the lid of a clean, closed petri plate for several hours to collect discharged ascospores. The ascospores were taken up in sterile distilled water, and the suspension was adjusted to about 100 ascospores per droplet. A Burkard microapplicator was used to place the suspension on the exposed xylem of pruning wounds at the junction of the recently laid down and older xylem. Because ascospores are usually dispersed during rain, all sites were atomized with sterile distilled water before inoculation. Ascospore viability at the time of inoculation was always confirmed by placing droplets of inoculum on agar plates in the laboratory and examining them microscopically for germination 1 day later.

Fungicide treatments. Except in the nonchemical treatments, fresh wounds were hand-painted immediately with brush applications of either benomyl (Benlate 50W) at 0.2 lb per gallon (2.4%) of water (a.i. 10,000 ppm) or thiabendazole (Mertect 340F) at 2.84 or 5.68 fl oz per gallon (2.2 or 4.4%) of water (a.i. 10,000 or 20,000 ppm).

Assessment of inoculation results. The incubation period from the time of experimental mycelial inoculation of fresh grapevine wounds until symptom expression extends for 1–3 yr (2). Reisolation from the pruning wounds after an 18-mo incubation was therefore necessary to determine the incidence of infection. The spurs bearing the treated wounds were removed from the vine and brought to the laboratory, where isolations were made as previously described (2).

Experimental design. *Duration of wound susceptibility and protective effect of benomyl.* Groups of two or three adjacent arms on a vine constituted an experimental plot; 20 replicates of each plot were scattered in a completely randomized design on 30 vines, for a total of 50 sites per treatment. Of the six treatments (Table 1), four involved inoculation at 0, 8, 14, or 22 days after the original pruning date of 15 February 1977 and one involved application of benomyl (10,000 ppm) to fresh wounds a few hours before inoculation. There was one noninoculated control treatment.

Effect of grapevine wood age and wound age on infection. Groups of two or three adjacent arms on a vine constituted an experimental plot; eight replicates of each plot were scattered on 10 vines in a completely randomized design, for a total of 20 sites per treatment. All four treatments (Table 2) were applied on 15 February 1977.

Protective effect of thiabendazole paint. Sixteen half vines were used, and each of the three treatments (Table 3) was randomized on each half vine, for a total of 16 sites per treatment. All treatments were applied on 7 April 1978.

RESULTS

The level of natural Eutypa dieback infection after February pruning was measured by culturing from 50 wounds that were also made on 15 February 1977 and not inoculated. *Eutypa* reisolation after 18 mo was 15% (Table 1), probably because natural inoculum dispersed during the rains that fell 6 days after pruning. With 41 infected arms vs. 15 expected, inoculation on day 0 without the use of fungicide paint was obviously the significantly different population. There were no significant differences among the other five treatments.

The data in Table 2 show that February inoculations made on 1-yr-old wood resulted in significantly fewer infections than comparable inoculations made on 2-yr-old or older wood.

There was no significant difference in infection rate between the number of arms treated with either 10,000 or 20,000 ppm thiabendazole paint (Table 3). The chi-square value, however, was highly significant for no fungicide vs. thiabendazole paint.

DISCUSSION

The data further confirm the role of *E. armeniacae* in the etiology of "dieback" of grapevines. Ascospore inoculations led to infection in the same manner as mycelial inoculations (2), although doses of 100 spores applied to fresh wounds did not result in 100% infection, based on the

Table 1. Duration of susceptibility of grapevine pruning wounds and efficacy of 10,000 ppm benomyl as wound protectant against *Eutypa* infection[a]

	Number of *Eutypa*-infected arms			Chi-square			
					Required		df
Treatment	**Expected**	**Observed**	**Total**	**Observed**	**0.05**	**0.01**	
No fungicide paint; inoculated day 0	15.3	41(83.7%)	49				
No fungicide paint; inoculated day 8	15.0	15(31.3%)	48				
No fungicide paint; inoculated day 14	14.0	10(22.2%)	45				
No fungicide paint; inoculated day 22	15.6	12(24.0%)	50				
No fungicide paint; not inoculated	15.0	7(14.6%)	48				
Benomyl paint (10,000 ppm); inoculated day 0	15.3	5(10.2%)	49				
Total		90	289	81.92**[b]	11.07	15.09	5

[a]Fifty wounds made 15 February 1977 on 2-yr-old wood were inoculated with about 100 ascospores on the day indicated; isolations were made 18 mo later.
[b]** = Statistically significant at $P \leqslant 0.01$.

Table 2. Effect of grapevine wood age and wound age on *Eutypa* infection[a]

	Number of *Eutypa*-infected arms			Chi-square, 1 df		
					Required	
					$P \leqslant 0.05$	3.84
Treatment	**Expected**	**Observed**	**Total**	**Observed**	**0.01**	**6.64**
Fresh wound on 1-yr-old wood	12.5	8(40%)	20			
Fresh wound on 2-yr-old wood	12.5	17(85%)	20			
Total		25	40	8.75**[b]		
Fresh wound on 1-yr-old wood	12.0	8(40%)	20			
Fresh wound on 3- to 4-yr-old wood	21.0	16(80%)	20			
Total		24	40	6.77**		
Old wound on 2-yr-old wood	10.5	4(20%)	20			
Fresh wound on 2-yr-old wood	10.5	17(85%)	20			
Total		21	40	17.04**		

[a]Wounds made 15 February 1977 on wood of indicated age were inoculated with about 100 ascospores on the day of pruning; isolations were made 18 mo later.
[b]** = Statistically significant at $P \leqslant 0.01$.

Table 3. Evaluation of two rates of thiabendazole paint as wound protectant against *Eutypa* infection[a]

Treatment	Number of *Eutypa*-infected arms			Chi-square			df
	Expected	Observed	Total	Observed	Required 0.05	Required 0.01	
No fungicide	6.7	12(75.0%)	16				
Thiabendazole 10,000 ppm	6.7	5(31.3%)	16				
Thiabendazole 20,000 ppm	6.7	3(18.8%)	16				
Total		20	48	11.47**[b]	5.99	9.21	2

[a]Wounds made 7 April 1978 on 2-yr-old wood were inoculated with about 100 ascospores on the day of treatment; isolations were made 14 mo later.

[b]** = Statistically significant at $P \leqslant 0.01$.

reisolation criteria we used. Reexamination of apricot tree susceptibility data of Ramos et al (5), however, reveals a similar result with 100-spore inocula during late winter and spring. The results further confirm that under the conditions of this experiment, susceptibility of wounds of 2-yr-old grapevine wood made in late winter is significantly higher during the first week after pruning than 2 or 3 wk later.

Wounds made on 1-yr-old grape wood during late winter were significantly more resistant than comparable wounds made on older tissue. This appears to be in contrast to apricot (5), where recently differentiated wood exhibited higher susceptibility. In California vineyards, Eutypa dieback rarely appears in *V. vinifera* grapevines younger than 6 yr but can be severe in older plantings; this may be explained by the reduced susceptibility of younger wood and fewer heavy pruning cuts, coupled with the fact that symptoms generally do not appear until several years after inoculation.

The benomyl paint treatment conferred a high degree of protection to grapevine pruning wounds. Such a practice would be economically feasible because very little fungicide is required, although hand-painting the wounds would increase the labor cost. The procedure may well be justified in areas where Eutypa dieback results in reduced vineyard productivity because of missing or seriously weakened vines. Thiabendazole also had a significant effect on infection levels but did not provide a level of control as high as that of benomyl; even the concentration of 20,000 ppm (a.i.) did not measure up to its efficacy against the same pathogen on apricot (3,4).

ACKNOWLEDGMENTS

We wish to thank W. Ishisaka and E. P. Vilas, Jr., staff research associates at the University of California, Davis, for technical assistance.

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