Fungicidal Control of *Eutypa armeniacae* Infecting Concord Grapevine in Michigan

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ABSTRACT

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Field trials were conducted at four different dates in the winter and early spring of 1979–1980 and also in 1981 in a commercial vineyard at Lawton, MI. In the 1979–1980 trials, a benomyl 50WP spray of 4.8 g/L water (0.5 L per vine) gave significant control of ascospore infection by *Eutypa armeniacae* when pruning wounds on 2-yr-old wood were inoculated either on the day of pruning and spraying or 14 days later. A lower rate of benomyl 50WP, two rates of captafol 4F, and a biological control treatment of *Fusarium lateritium* gave little or no control. Pruning wounds declined in susceptibility over the 14-day period. No differences in susceptibility to infection were noted among the four dates of pruning. In the 1981 trial, benomyl 50WP showed significant disease control at 1.2 and 9.6 g/L water compared with water-treated control vines.

Eutypa dieback, also referred to previously as "dying arm" (12) or "dead arm" (14), is an important disease of grapevine worldwide, causing cankering and necrosis of woody tissue. It had been reported in New York (1), Japan (6), Michigan (18), New Zealand and Australia (5), Ontario (4), California (9), Greece (7), and Mexico and France (17). *Eutypa armeniacae* Hansf. & Carter has only recently been proven to be the causal agent (10) after early studies that had associated the leaf and cane spotting organism *Phomopsis viticola* with that role (19).

In Michigan, Eutypa dieback has been estimated to occur in about 10% of the mature grapevines (A. Trese, *unpublished*), and in California, disease levels of 81% have been reported (12).

Fungicidal control studies in the past have demonstrated that benomyl painted on fresh pruning wounds of apricot (8) and grapevines (11) reduced establishment of infection by *E. armeniacae* ascospores in California. Benomyl and captafol incorporated in agar at 10 μg/ml inhibited germination of *E. armeniacae* ascospores by 65 and 100%, respectively (A. Trese, *unpublished*).

Macroconidial suspensions of *Fusarium lateritium* Nees. were effective against *E. armeniacae* on apricot in field trials in Australia (3). Differences in pruning practices between California and Michigan preclude the use of fungicidal paints on pruning wounds in Michigan. Therefore, we have initiated field evaluations of fungicidal sprays for the control of Eutypa dieback.

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MATERIALS AND METHODS

Field trials, 1979–1980. To examine the possibility of using fungicides on fresh pruning wounds of grapevine (*Vitis labrusca* L. 'Concord') to prevent establishment of *E. armeniacae* ascospores, fungicide trials were conducted on 5 December 1979 and repeated on 15 January, 13 March, and 17 April, 1980 in a 10-yr-old commercial vineyard located about 5 km south of Lawton, MI. No evidence of previous infection by *E. armeniacae* could be found in this study or by A. Trese (*unpublished*) in this vineyard. The vines had been pruned since establishment into a bilateral cordon system in order to facilitate mechanical harvesting.

Twenty pruning wounds per vine were made just above a node or branch on 2-yr-old wood on each date mentioned previously. Any vines so small that 20 pruning wounds on 2-yr-old wood could not be obtained were eliminated from the trials.

After pruning, on the same day, each vine was sprayed with 0.5 L of a suspension (equivalent to 741.3 L/ha [79.3 gal/acre]) of either benomyl 50WP at the rate of 1.2 or 4.8 g/L (1 or 4 lb/100 gal) water, captafol 4F at the rate of 10 or 20 ml/L (4 or 8 qt/100 gal) water, or a water control.

In the March and April trials, a sixth treatment of 500 macroconidia per pruning wound of *F. lateritium* suspended in 5 μL sterile distilled water (SDW) was applied. The *F. lateritium* culture was provided by M. V. Carter (Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia). This inoculum was scraped off cultures growing on Difco potato-dextrose agar (PDA) and suspended in SDW. Concentrations were determined using a hemacytometer. There were five vines per treatment in a completely randomized plot design.

After treatment, on the same day, 10 of the 20 pruning wounds per vine were each inoculated with 500 ascospores of *E. armeniacae* suspended in 5 μL SDW. The other 10 pruning wounds were inoculated 14 days later when possible (weather permitting). In the January trial, however, it was necessary to make the second inoculation 35 days after pruning instead of 14 days because of the absence of above-freezing temperatures during that period. An inoculum level of 500 ascospores was chosen because Trese et al (18) were able to obtain an overall infection rate of 14.3% on 2-yr-old wood only when inoculated at various times of the season with 250 ascospores.

Ascospores for these inoculations were obtained by soaking mature stroma of *E. armeniacae* in SDW for 10 min. After 1 day, freehand sections of the stroma were made with a razor blade, cutting through numerous perithecia and suspending the freehand sections in a few milliliters of SDW. After a few seconds of shaking followed by a 1-hr wait, the suspension was again shaken and the ascospore concentration was determined with a hemacytometer. In no case were spores other than those of *E. armeniacae* seen during microscopic examination of the spore suspension; and ascospore germination was consistently between 90 and 97%.

In the December 1979 and January and March 1980 trials, the temperature was always between 0 and 5 C during pruning, spraying, and inoculation procedures; in the April 1980 trial, the temperature was about 10 C during these events.

Isolations and identifications of *E. armeniacae* taken from the pruning sites were made 9–13 mo after inoculation by the method of Trese et al (18). Each cane was cut off 6–10 cm below the original pruning wound. Through use of aseptic

techniques, the canes were then split lengthwise and 10 small wood chips were removed from 1–5 cm below the pruning wound and placed on 2% PDA amended with 100 μg/ml streptomycin sulfate. After 3–6 days, any fungal colonies resembling *E. armeniacae* were transferred to fresh potato-glucose agar (an extract of 200 g potatoes, 8 g glucose, and 20 g agar in 100 ml distilled water). These plates were placed under cool-white fluorescent light (GE F15T8CW) and soft black light (GE F30T8SB) with a 14-hr photoperiod. After about 1 mo, the presence of *E. armeniacae* was confirmed microscopically by the presence of scolecospores.

Field trials, 1981. Based on preliminary results from the first year trials, the second year trials were begun on 14 February 1981. The same vineyard was used as in the 1979–1980 trials. The trial was conducted as a 4 × 4 (16) factorial experiment. Benomyl 50WP at the rate of 1.2, 4.8, or 9.6 g/L (1, 4, or 8 lb/100 gal) water and a water control was applied after pruning as before. There were only 15 pruning wounds per vine in this trial, however. The pruning wounds were then inoculated as before on day 1, 14, 30, or 53 after pruning and spraying to determine how long fungicidal protection was needed. The temperature was between −2 and 5 C for the first three inoculation periods and 22 C for the fourth inoculation period. There were three randomly selected vines per fungicide treatment-inoculation date combination. Isolations and identification of *E. armeniacae* was made as before, 6–7 mo after pruning and spraying.

RESULTS

Field trials, 1979–1980. Table 1 shows cumulative totals of *E. armeniacae* infections over all four blocks (time periods). The first part of the table shows the data for the inoculation done on the day of pruning and spraying. The second part shows the data for the second inoculation (14 days after pruning and spraying). The data from block 2 are excluded here because the second inoculation was done at 35 days after pruning and spraying instead of at 14 days, because of continuous subfreezing weather. The high rate of benomyl was the only treatment that gave consistent reduction of infection compared with the untreated control for all time intervals between fungicide spraying and inoculation according to the chi-square test. There was no significant difference in infection (susceptibility) of the grape wood inoculated at these different times of the dormant season.

Table 2 contains a Duncan's multiple range test of the differences between the treatments at both inoculation times, excluding the *F. lateritium* treatment. There were significant differences from the control with both rates of benomyl and with 20 ml/L captafol. The 4.8 g/L rate of benomyl was the only treatment to give control at the 1% level of significance. The *F. lateritium* treatment was excluded from Table 2 to facilitate analysis (it was only in two, rather than all four of the blocks) and because it gave less control than the chemical treatments (Table 1).

Field trials, 1981. There was a significant difference among the fungicide treatments but not among time intervals between fungicide treatment and inoculation (Table 3). Duncan's multiple range test revealed a statistically significant reduction in infection due to benomyl 50WP at 1.2 and 9.6 g/L compared with the control.

DISCUSSION

Clearly, a benomyl spray works well in preventing establishment of *E. armeniacae* on pruning wounds. The most cost-effective rate, however, has not been defined. In 1979–1980, both the 1.2 and 4.8 g/L rate of benomyl gave significant control, whereas in 1981, 1.2 g/L was more effective than 4.8 g/L but not as effective as 9.6 g/L.

The higher benomyl rates were more successful in preventing infections introduced 2 wk or later after pruning and spraying. This is apparent when one observes that in the 1979–1980 trials, the 4.8 g/L rate of benomyl showed statistically significant control at the second inoculation time (Table 1), whereas the low rate did not.

A preventative benomyl spray should prove useful as a control measure for Eutypa dieback in Michigan. Spraying with a hand-held sprayer or perhaps a

Table 1. Effects of fungicides or a biological control agent on infection of Concord grapevine pruning wounds inoculated with *Eutypa armeniacae* at Lawton, MI, in 1979–1980[a]

Treatment	No. pruning wounds inoculated	Infected[b] (%)	Reduction of infection compared with control (%)	X^2 vs. control	Significance level of X^2 value (P =)
Inoculation on day of pruning and spraying[c]					
Control	173	37.0	...	...	...
Benomyl 50WP, 1.2 g/L	186	17.7	52.0	15.89	<0.005
Benomyl 50WP, 4.8 g/L	179	10.0	74.3	35.93	<0.005
Captafol 4F, 10 ml/L	185	23.2	37.2	7.42	<0.01
Captafol 4F, 20 ml/L	187	20.9	43.6	10.68	<0.005
F. lateritium	93	23.7	36.0	4.33	<0.01
Inoculation 14 days after pruning and spraying[d]					
Control	138	11.6	...	...	...
Benomyl 50WP, 1.2 g/L	133	7.5	35.1	<1	>0.1
Benomyl 50WP, 4.8 g/L	131	3.1	73.7	5.94	<0.01
Captafol, 10 ml/L	135	10.4	10.5	<1	>0.1
Captafol, 20 ml/L	138	10.2	12.5	<1	>0.1
F. lateritium	89	7.9	32.1	<1	>0.1

[a] This experiment was conducted in a healthy 10-yr-old commercial vineyard of *Vitis labrusca* L. 'Concord.' Twenty pruning wounds were made per vine on 2-yr-old wood. There were five vines per treatment. Each vine except the *F. lateritium*-treated vines was sprayed after pruning with 0.5 L of its respective treatment. Each wound on the *F. lateritium*-treated vines was inoculated with 500 macroconidia of *F. lateritium*. Ten of the 20 pruning wounds per vine were inoculated with 500 ascospores of *E. armeniacae* in 5 μL distilled water on the day of pruning and spraying. The other 10 pruning wounds per vine were inoculated as described above on day 14 after pruning and spraying. The four dates of pruning and spraying were 5 December 1979 (block 1), 15 January 1980 (block 2), 13 March 1980 (block 3), and 17 April 1980 (block 4). Positive infections were determined by tissue isolations onto PDA 9–13 mo after treatment.

[b] Analysis of variance yielded an F value of 5.38 (P = 0.01) for treatments, an F value of 11.86 (P = 0.004) for inoculation time, and an F value of 1.99 (P = 0.169) for blocks (pruning dates).

[c] All four blocks combined.

[d] Block 2 was excluded because the second inoculation was done at day 35 instead of at day 14 because of subfreezing conditions.

Table 2. Duncan's multiple range test of data from fungicidal control trials on Concord grapevine pruning wounds inoculated with *Eutypa armeniacae* at Lawton, MI, in 1979–1980[w,x]

Treatment	Infection (%)
Control	24.08 a[y]
Benomyl 50WP, 1.2 g/L	14.48 bc
Benomyl 50WP, 4.8 g/L	7.49 c[z]
Captafol 4F, 10 ml/L	18.51 ab
Captafol 4F, 20 ml/L	15.66 bc

[w] The four dates of pruning and spraying were 5 December 1979, 15 January 1980, 13 March 1980, and 17 April 1980. The values shown are the means derived from the four pruning and spraying dates at both inoculation times.

[x] Each vine was sprayed after pruning with 0.5 L of its respective treatment. Ten of the 20 pruning wounds per vine were inoculated on the day of pruning and spraying with 500 ascospores of *E. armeniacae* in 5 μL distilled water. The other 10 pruning wounds per vine were inoculated as described previously on day 14 (or 35 in the case of block 2) after pruning and spraying.

[y] Treatments followed by a common letter are not significantly different at P = 0.05.

[z] Significantly different from control at P = 0.01.

Table 3. Effects of three benomyl spray rates and time interval between spraying and inoculation on infection of Concord grapevine pruning wounds with *Eutypa armeniacae* at Lawton, MI in 1981[a]

Rate of benomyl 50WP (g/L)	Interval between pruning + spraying and inoculating (days)	No. pruning wounds inoculated	Pruning wounds infected (%)
0	1	42	14.3
0	14	41	12.2
0	30	42	7.1
0	53	40	2.1
1.2	1	40	5.0
1.2	14	41	4.9
1.2	30	41	2.4
1.2	53	43	0.0
4.8	1	44	9.1
4.8	14	41	2.4
4.8	30	44	6.8
4.8	53	41	2.4
9.6	1	44	0.0
9.6	14	43	4.7
9.6	30	42	2.4
9.6	53	43	0.0
0	...	165	9.1 y[b,c]
1.2	...	165	3.0 z
4.8	...	170	5.3 yz
9.6	...	172	1.7 z
...	1	170	7.1[b]
...	14	166	6.0
...	30	169	4.7
...	53	167	1.2

[a] This experiment was conducted on a healthy 11-yr-old commercial vineyard of *Vitis labrusca* L. 'Concord.' The date of pruning and spraying was 17 February 1981. Fifteen pruning wounds were made per vine on 2-yr-old wood. There were three vines per treatment-inoculation date combination. Each vine was sprayed with 0.5 L of its respective treatment. Inoculations were made by placing 500 ascospores of *E. armeniacae* in 5 μL sterile distilled water on each pruning wound. Positive infections were determined by tissue isolations onto PDA 6–7 mo after treatment.

[b] Analysis of variance (4 × 4 factorial) yielded an F value of 3.16 ($P = <0.05$) for treatments. However, an F value of 1.99 for time interval (days) between fungicide treatment and inoculation was not significant ($P = >0.1$).

[c] Treatments followed by a common letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

small power sprayer should be easier than applying a paint, which was an effective treatment in California (11). In California, there are only a few pruning wounds on a vine. In Michigan, 40 or 50 cuts per vine are usual.

The *F. lateritium* treatment was not effective, even though the organism consistently became established in more than half of the pruning wounds to which it was inoculated (E. Gendloff, *unpublished*). Apparently, *F. lateritium* is not inhibitory to the Michigan isolate of *E. armeniacae*. In the stems that were inoculated with *F. lateritium*, more than half of the stems from which *E. armeniacae* was isolated also contained *F. lateritium* (E. Gendloff, *unpublished*). This was true in all trials in which *F. lateritium* was included at either time of *E. armeniacae* inoculation.

In our study, susceptibility of pruning wounds decreased with time after pruning. This change in susceptibility with time was found to be highly significant in the first year trials. Although the differences in susceptibility over time were not statistically significant in the second year trials, the trend was consistent. Similar findings have been reported on apricot in Australia (2) and California (15), and on grape in California (13).

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