Effects of Winter and Spring Pruning and Postinoculation Cold Weather on Infection of Grapevine by *Eutypa armeniacae*

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ABSTRACT

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Field experiments involving pruning mature *Vitis labrusca* 'Concord' grapevines and inoculation of the pruning stubs with *Eutypa armeniacae* were conducted near Lawton, MI. Eight pruning and inoculation dates were tested in 1978 and 1979. Temperature and rainfall were monitored for 2 wk following pruning and inoculation. When 1-, 2-, or 3-yr-old wood was pruned and inoculated the same day, age had no significant effect on susceptibility. Mean percentages of infection (all dates of inoculation included) for 1-, 2-, and 3-yr-old pruned wood were 13.3, 14.3, and 15.2%, respectively. Uninoculated control vines, which were pruned at the same time as the inoculated vines, had a small amount of infection (0.5–2.0%). Control vines probably were infected by windblown ascospores from diseased vineyards 0.8 km away. Pruning 22 February and 30 March 1979 resulted in significantly ($P = 0.05$) greater percent infection (42 and 20%, respectively) than the six other dates (9 March 1978 through 20 December 1978), which ranged from 2 to 11% infection. In general, the greater the percentage of the 2-wk period following pruning and inoculating that the temperature was 5 C or lower the less was the resultant percentage of infection.

Additional key words: epidemiology, Eutypa dieback.

The role of *Eutypa armeniacae* Hansf. and Carter as a pruning wound invader and incitant of Eutypa dieback of grapevine has been documented under conditions in California (2), Michigan (8), and New York (4). Studies in Michigan (8) and New York (4) have shown that ascospores are liberated by small amounts of rainfall when the temperature is >0 C. Time of abundant inoculum availability coincided with the time of pruning grapevines in the northeastern United States (4,8).

Petzoldt et al (5) found that small, propagated grapevines grown under greenhouse and lathhouse conditions in California were most susceptible in December and least susceptible in March. The authors suggested that vines in California should be pruned in late February and March to minimize natural infection. They also indicated that suceptibility of pruned vines decreased after 2 wk. We also found a trend of decreasing susceptibility with increasing time in days between pruning and inoculation for as long as 56 days under low temperature conditions (8). Since our report, there has been no published study involving inoculation of pruned mature vines with *E. armeniacae* under cold climate winter-spring conditions, nor has there been a detailed evaluation of weather effects following pruning and inoculation of these sites.

The objectives of this research were to ascertain the relative susceptibilities of pruning wood of different ages, to determine the effects of pruning time upon susceptibility of pruned grape stubs,

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and to examine possible relationships between infection and temperature and rainfall patterns during the 2-wk period following inoculation of pruning wounds.

MATERIALS AND METHODS

An 8-yr-old planting of *Vitis labrusca* L. 'Concord' vines was chosen as the site for this experiment. This vineyard was located south of Lawton, MI, and it was 0.8 km from other vineyards. Several previous careful inspections of the vines for symptoms during spring growth (the best time to detect Eutypa dieback disease symptoms) did not reveal any infected vines in the planting.

On eight dates during the autumn, winter, and spring during 1978 and 1979, pruning cuts were made on 1-, 2-, and 3-yr-old canes. Each site was immediately (within 1 or 2 hr) inoculated with 5 μl of a suspension containing 250 ascospores of *E. armeniacae*, prepared as previously described (8). Prior to inoculation, the pruning stubs were lightly misted with water from a DeVilbis atomizer to simulate rainfall that precedes or coincides with inoculum liberation. Control vines were similarly pruned, tagged, and each stub was 'sham-inoculated' with 5 μl of water. Sets of five vines were pruned on each date with a total of 50–75 pruning cuts on the five vines. The test plot was arranged in a completely randomized design (1). Inoculations made were as follows: 9 March, 30 March, 20 April, 18 May, 29 November, and 20 December in 1978 and 22 February and 20 March in 1979. On each date, the temperature was above freezing at the time of inoculation, so that the inoculum would not immediately freeze.

A weather station 0.8 km from the vineyard was used to monitor conditions after inoculation. The station contained a sheltered hygrothermograph (Bendix Corp., Baltimore, MD 21204) and a recording rain gauge (Weather Measure Corp., Sacramento, CA 95841) both located 1 m above ground level.

One year after inoculation, tissue isolations were made from the inoculated pruning stubs onto potato-dextrose agar (PDA) to assay for infection. Each pruning stub was excised several centimeters below the inoculation point and transported in plastic bags to East Lansing. Cane pieces were split longitudinally and 10 small segments of woody tissue were aseptically removed from the area below the inoculation site. Chips were plated out in petri plates containing Difco PDA (Difco Laboratories, Detroit, MI 48232) amended with 100 μg of streptomycin sulfate per milliliter. The plates were examined after incubation for 4–7 days at room temperature and all fungal colonies resembling *Eutypa* were transferred to fresh potato-glucose agar (8). These plates were incubated under cool-white fluorescent light (General Electric 15T8 CW) and soft black light (General Electric F30T8 SB) with a 14-hr day length. After ~2 wk *E. armeniacae* cultures were identified by the presence of scolecospores and characteristic mycelial growth.

RESULTS

Results of field inoculations are shown in Table 1. Chi-square analysis (1,7) was done to test three null hypotheses: (i) H_0: Infection is independent of inoculation; (ii) H_0: Infection is independent of age of wood at inoculation; (iii) H_0: Infection is independent of the pruning and inoculation date. In all cases, higher levels of infection developed after inoculation compared to the natural 'background' infection. At $P = 0.05$, null hypotheses i and iii were refuted. In other words, i, infection was not independent of inoculation; ii, infection was independent of the age of wood inoculated; and iii, infection was not independent of the pruning and inoculation date. Mean percentages of infection for all inoculation dates for 1-, 2-, and 3-yr-old inoculated pruning stubs, respectively, were 13.3, 14.3, and 15.2%. The mean percentages of uninoculated control 1-, 2-, and 3-yr-old pruning stubs, respectively, were 1.0, 0.5, and 2.0%. Percentages of infection for the 22 February 1979 (42%) and 30 March 1979 (20%) inoculation dates were significantly ($P = 0.05$) higher than those for other inoculation dates, which ranged from 2 to 11% infection.

A summary of the 2 wk of temperature and rainfall patterns following each inoculation date are shown in Table 2. Correlation coefficients were calculated between percentage of pruning wounds infected resulting from each inoculation and the percentage of time that temperatures were at various levels for a 2-wk period following pruning and inoculation. Also, correlation coefficients were calculated for percentage infection resulting from each inoculation date versus rainfall factors. Because of an abnormally high level of infection (42%) that resulted from the 22 February 1979 inoculation date (a statistical "outlier" for an unexplained reason), correlation coefficients were run two ways. One set was run including, and one was run excluding, the data collected on that date. Table 2 shows that correlation coefficients between various temperature levels and rainfall patterns during the 2-wk period following pruning and inoculation are too low to show any

TABLE 1. Effect of age of 'Concord' grape wood and date of pruning upon infection by *Eutypa armeniacae* ascospores in a mature vineyard at Lawton, MI, 1978–1979

	Inoculated pruning wounds[a,c] Age of wood pruned			Overall infection[b]	Uninoculated pruning wounds[c] Age of wood pruned			Overall infection[b]
Pruning date	1 yr	2 yr	3 yr	(%)	1 yr	2 yr	3 yr	(%)
1978								
9 March	0/14[d]	1/29	0/3	2	1/17	0/31	0/7	1.7
30 March	1/26	7/40	0/5	11	0/16	1/29	1/4	4.0
20 April	2/19	5/40	0/3	11	0/15	0/28	0/4	0.0
18 May	5/25	2/37	0/3	11	0/22	0/31	0/2	0.0
29 November	...[e]	2/26	1/23	6	...	0/26	1/23	2.0
20 December	...	0/23	1/24	2	...	0/19	0/27	0.0
1979								
22 February	5/16	12/24	10/24	42	0/16	0/23	0/19	0.0
30 March	4/20	6/26	5/27	20	0/18	0/24	0/25	0.0
Totals[f]	17/128 (13.3%)	35/245 (14.3%)	17/112 (15.2%)	69/485 (14.2%)[g]	1/104 (1.0%)	1/211 (0.5%)	2/111 (2.0%)	4/426 (0.9%)[g]

[a] Pruning cuts were made and the sites immediately inoculated with a spore suspension of 250 ascospores of *E. armeniacae* in 5 μl of water.
[b] The overall infection by date includes all three ages of wood that were pruned and inoculated.
[c] Numerator denotes number of inoculation sites infected with *E. armeniacae* and the denominator denotes the number of sites, either inoculated or uninoculated.
[d] No inoculations were made on controls marked for 1-yr-old canes on these dates.
[e] Total number of infected pruning sites per age category of wood pruned.
[f] Chi-square tests were run to test the following null hypothesis: (i) H_0: Infection is independent of inoculation. (ii) H_0: Infection is independent of age of wood inoculated. (iii) H_0: Infection is independent of date on which pruning and inoculation are done. At $P = 0.05$ i and iii were refuted. For null hypothesis i, ii, and iii, the observed χ^2 values were, respectively, 54.33 (at 1 df), 0.17 (at 3 df), and 58.7 (at 7 df). The corresponding required values were, respectively, 7.88, 5.99, and 5.99.

TABLE 2. Correlation coefficients between infection of pruning wounds of 'Concord' grape and rainfall and temperature for a 2-wk period after inoculation with *Eutypa armeniacae*

Pruning date	Pruning wounds infected (%)	Percentage of time temperature was at: <0 C	<5 C	>0 C	>5 C	>10 C	No. of rains	Amount of rain (cm)	Total duration of rain (cm)
1978									
9 March	2	24	86	77	14	5	2	2.1	14
30 March	11	0	30	100	68	37	5	1.2	17
20 April	11	6	28	93	71	31	4	0.28	16
18 May	11	0	2	100	98	39	1	1.38	3
29 November	6	51	94	57	8	3	2	2.85	24
20 December	2	56	95	50	4	3	4	2.50	25
1979									
22 February	42	22	84	79	21	2	3	2.95	30
30 March	20	15	67	86	35	15	6	3.72	33
R^a		−0.267	0.023	0.252	0.027	−0.119	0.166	0.326	0.464
R^b		−0.605	−0.489	0.616	0.509	0.508	0.552	0.135	0.248

[a]Correlation coefficient with data from the 22 February 1979 pruning date included.
[b]Correlation coefficient with data from the 22 February 1979 pruning date excluded.

meaningful relationship. However, if the data from 22 February 1979 are excluded, some interesting correlation coefficients result. For example, levels of infection correlate inversely with the percentage of time the temperature was below 0 or 5 C ($R = -0.605$ and -0.489, respectively). Conversely, levels of infection correlate positively with the percentage of time that the temperature was above 0, 5, or 10 C ($R = 0.616$, 0.509, and 0.508, respectively). Although these R values are not very high, they do give an indication that near freezing or subfreezing temperatures after pruning result in very low infection and that infection is correspondingly higher when temperatures are still fairly low; eg, 5–10 C during the spring, when much of the pruning has historically been done.

The only correlation coefficient relative to rain that was possibly meaningful was that of number of rain events following pruning and inoculation, that being 0.508, as a result of excluding the data of 22 February 1979. Of course, it must be reiterated that rainfall is absolutely necessary for natural ascospore inoculum liberation and infection of pruning wounds (4,8). There is the inherent risk of missing some possible relationship between the very high infection (42%) and the environmental conditions recorded, or possibly some other condition of which we are unaware. We have not been able to adequately explain this very high infection level.

DISCUSSION

In general, the data indicate that little infection results when pruning is done during extended periods of cold weather; ie, at or below freezing or at 5 C or less for 2 wk after pruning cuts are made. The resulting amount of infection was minimal; ie, 2–6%. Extended periods of temperatures at or below freezing usually occur during December and January in Michigan and in most northeastern states and eastern Canada. However, a January thaw may alter the situation. Rains and warmer temperatures associated with a January thaw could lead to increased infection of recently pruned vines. Although previous studies have shown that small amounts of rainfall are necessary to release ascospore inoculum, our data do not indicate any real effect of frequency or amount of rain upon infection after inoculation has occurred.

It is possible that physiological changes in vine wood status may affect susceptibility to infection. For example, the onset of sap flow in the spring might make a more desirable site for infection and fungal growth or conversely, actively "bleeding" pruning wounds in late spring could create conditions unfavorable for infection.

Although Ramos et al (6) showed that *E. armeniacae* inoculum is at a low ebb in apricot orchards in California from November through early January, and Petzoldt et al (5) showed that grapevine susceptibility may be at its lowest during late February or March, our spore trapping and inoculation data indicate that there is no definite "safe" time to prune vines in Michigan. Consequently, protectant fungicidal treatment of freshly pruned vines is probably the most promising method of control. Moller and Kasimatis (3) showed that a 10 g/L (a.i.) suspension of benomyl painted on large pruning cuts gives reasonably good protection. While the California pruning system consists of making a few large cuts on a vine, the type of pruning in Michigan and the rest of the northeastern United States and eastern Canada involves making about 50 cuts per vine. The majority are on 1-yr-old canes, but a few 2- and 3-yr-old canes were also cut. We have shown that there is no significant difference in susceptibility of the age of cane wood pruned (this agrees with the findings of Petzoldt et al [5]), and our preliminary data (E.H. Gendloff and D.C. Ramsdell, *unpublished*) show that the causal fungus does not grow more than 2 cm down into a pruning stub after one season. It is possible that the majority of the infected pruning stubs would be pruned off the next year before the pathogen was likely to enter the trunk or arm.

Although painting benomyl on a small number of pruning wounds on a vine may be feasible in California, this method is not feasible in Michigan because of the large number of pruning cuts made on a vine. We have field experiments in progress that indicate that benomyl sprays at high concentrations (ie, 5.5 or 22.0 g/L a.i.) can give significant disease prevention if sprays are applied soon after pruning and before subsequent rainfall (E.H. Gendloff and D.C. Ramsdell, *unpublished*).

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