Eutypa Dieback of Grapevine: Seasonal Differences in Infection and Duration of Susceptibility of Pruning Wounds

C. H. Petzoldt, W. J. Moller, and M. A. Sall

Graduate research assistant, extension plant pathologist, and assistant professor of plant pathology, respectively, Department of Plant Pathology, University of California, Davis 95616.
Portion of an M.S. thesis submitted to the University of California by the senior author.
Accepted for publication 30 October 1980.

ABSTRACT

Petzoldt, C. H., Moller, W. J., and Sall, M. A. 1981. Eutypa dieback of grapevine: Seasonal differences in infection and duration of susceptibility of pruning wounds. Phytopathology 71:540-543.

Grapevines growing in Davis, CA, were pruned at three different times (19 December 1978, 6 February 1979, and 12 March 1979) and wounds on 1-yr-old wood were inoculated with 0, 100, or 1×10^3 ascospores of *Eutypa armeniacae* at weekly intervals after pruning. The wounds were most susceptible to infection in December and least susceptible in March. Wounds were susceptible for a longer period of time after pruning in December than after pruning in March. Wound size and relative position of a wound on a vine did not have a significant effect on infection after inoculation. In most cases, more infections resulted when wounds were inoculated with 1×10^3 ascospores than when 100 ascospores were used. These findings suggest that pruning in late February or early March may be desirable to avoid Eutypa dieback in California regions where the disease is most prevalent.

Additional key words: apricot dieback, dying arm of grapevine.

A fungal dieback of apricot trees has been recognized since the 1930s (15); the sexual stage of the causal fungus subsequently was determined to be *Eutypa armeniacae* Hansf. and Carter (1). It also has been found to be a pathogen on European plum (*Prunus domestica* L.) (1), western chokecherry (*P. demissa* L.) (4), *Ceanothus* spp. (6,12), grapevine (*Vitis vinifera* L.) (9), and manzanita (*Arctostaphylos* sp. Adans.) (W. J. Moller and C. H. Petzoldt, *unpublished*).

E. armeniacae, first recognized as a saprophyte on grapevine in Australia in 1957 (2), also was found to be saprophytic on grapevine in California in 1968 (7), and subsequently was implicated as a cause of a "dying arm" condition of grapes (3,8,11). Pathogenicity was not confirmed until 1978 by Moller and Kasimatis (9). Confusion with a disease caused by *Phomopsis viticola* Sacc. delayed the recognition of Eutypa dieback symptoms (9). Symptoms of Eutypa dieback are best observed in early spring and include stunted, yellowed shoots with reduced leaf size and leaf cupping and necrosis (11). Invariably, faint vascular streaking can be traced from the diseased shoot to cankered wood surrounding an old pruning wound where a perithecial stroma may be produced. Ascospores released from these perithecia during rainfall are the only known natural inoculum, and infection occurs after ascospores land on fresh pruning wounds (9).

In Australia, California, and recently in Michigan and New York, an annual ascospore release cycle has been observed (6,10,13,16). Large numbers of ascospores are released by rain in the fall. In California in midwinter, there is often a period when numbers of ascospores are low, despite an incidence of rainfall that would release large quantities of ascospores at other times of the year. In early spring a resumption of large releases of ascospores occurs with each rainfall. Since pruning of grapevines can take place during all of these periods, infection sites are available to the fungus throughout this time. It would appear that pruning in midwinter, when ascospore levels are low, might be the safest time in order to avoid inoculum. However, any definite assessment of the probability of infection at these times must include the possibility of seasonal variations in grapevine susceptibility to infection. Susceptibility of grape wood has been little studied, but the incidence of the disease in older vineyards and some recent data (10) suggest that wounds made on 1-yr-old wood are less susceptible than those on older wood.

Ramos et al (14) found that apricot trees are most susceptible to *E. armeniacae* in the fall, least susceptible in the spring, and of intermediate susceptibility during the winter.

One of the major problems in a study of grapevine susceptibility to Eutypa dieback is that the length of time from inoculation to appearance of the first visible symptoms may be 3 yr or more (9).

The purposes of this research were to develop a more rapid method of determining infection by *E. armeniacae*; to elucidate the relative susceptibility of wounds made in different seasons on 1-yr-old wood; to compare the duration of wound susceptibility in each season; to determine the effect of different inoculum levels; and to determine the degree of correlation between infection, the diameter of wound, and the relative position of the wound on the vine.

0031-949X/81/05054004/$03.00/0

MATERIALS AND METHODS

Inoculation and reisolation from first-year grape wood. One hundred cuttings of first-year grape (*V. vinifera* L., 'Grenache') wood were rooted in a heated rooting bench in January 1978. After sufficient root formation, the cuttings were potted. They were pruned just above a live bud on 6 February 1978 and immediately inoculated with 5-μl droplets of an ascospore suspension by using a Burkard microapplicator (Rickmansworth, Herts, England). Spore suspensions were adjusted to deliver 0, 5×10^3, 20×10^3, 35×10^3, or 50×10^3 ascospores per wound. Twenty vines were inoculated with each ascospore suspension. Wounds were sprayed lightly with sterile distilled water before inoculation to simulate rain, which normally accompanies inoculation in nature, and to insure an even distribution of the spores over the wound surface.

Ascospores were obtained from an active stroma by soaking the stroma for 1 hr in sterile distilled water, then suspending the stroma over a plastic petri dish. Ascospores were released from the stroma and deposited on the petri dish. They were collected in a droplet of sterile distilled water and were diluted to the desired concentrations by using a hemocytometer.

Germination percentage on water agar for ascospores from each suspension was 99–100%.

After inoculation, the vines were grown in a greenhouse for 6 wk at 25 C, then moved to a lathhouse for another 15 wk. At the end of this time, isolations onto 10% potato-dextrose agar (PDA) were made from four of the vines in each group. Five vines from each group were planted in the field and the remaining 11 vines from each group were left in pots in the lathhouse for observation.

Isolations were made from the vines by excising a length of wood from below the wound that included the margin of live and dead tissue. These sections were split longitudinally and surface disinfested with 0.5% sodium hypochlorite. Twenty-five chips of wood were taken from various areas along the margin of live and dead tissue near the original wound. These were placed on PDA and incubated for 4–6 days. The plates were then examined for the presence of the distinctive mycelium of *E. armeniacae* (Fig. 1). The mycelium in culture is characterized by an irregular margin, whitish cottony appearance, and tufted aerial growth habit. If any of the 25 chips was found to contain *E. armeniacae*, the wound was considered infected. Previous experience indicated that 25 chips were necessary to detect the presence of the fungus.

Seasonal differences in infection and duration of susceptibility of pruning wounds. In the fall of 1978, 1,440 first-year canes were selected in a 4-yr-old vineyard (*V. vinifera,* 'Thompson Seedless') in Davis, CA, and left unpruned. These were classified randomly into three groups that were pruned on 19 December 1978, 7 February 1979, and 12 March 1979, respectively. Within each group, wounds were inoculated with 100 or 1,000 ascospores or water only (as controls). Ascospore suspensions were prepared as previously described. All three inoculations were made on the day after pruning and at approximately 1-wk intervals for the following 3 wk. Thus, on the first day after pruning, 40 sites received the water control, 40 sites received 100 ascospores, and 40 sites received 1,000 ascospores. This process was repeated for days 7, 14, and 21 after each pruning. Inoculations were made as described earlier by using a Burkard microapplicator.

Isolations were made as previously described after an 8-mo incubation period. Wound diameter was measured and relative vertical position on the vine was recorded. Relative vertical position on the vine was designated by increasing numbers from the lowest to the highest site inoculated.

RESULTS

Inoculation and reisolation from first-year grape wood. Five months after inoculation of 1-yr-old grapevines, *E. armeniacae* was isolated from three of four vines inoculated with 5×10^3 or 20×10^3 ascospores each and from four of four vines inoculated with 35×10^3 or 50×10^3 ascospores each. *E. armeniacae* was not isolated

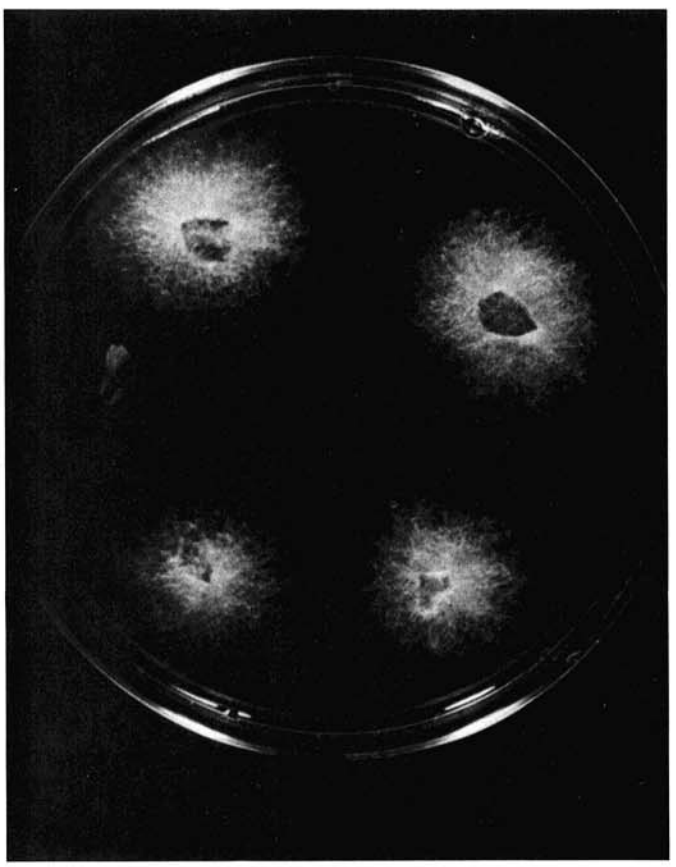

Fig. 1. Mycelium of *Eutypa armeniacae* on potato-dextrose agar.

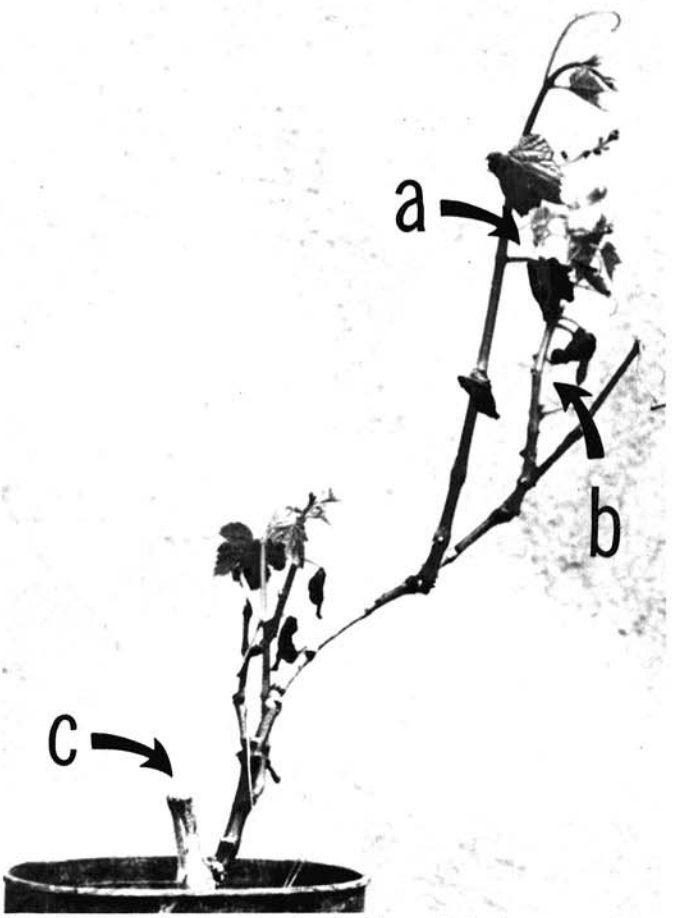

Fig. 2. One-year-old grapevines showing Eutypa dieback symptoms 14 mo after inoculation: (a) necrotic leaf tissue, (b) stunted internode, and (c) point of inoculation.

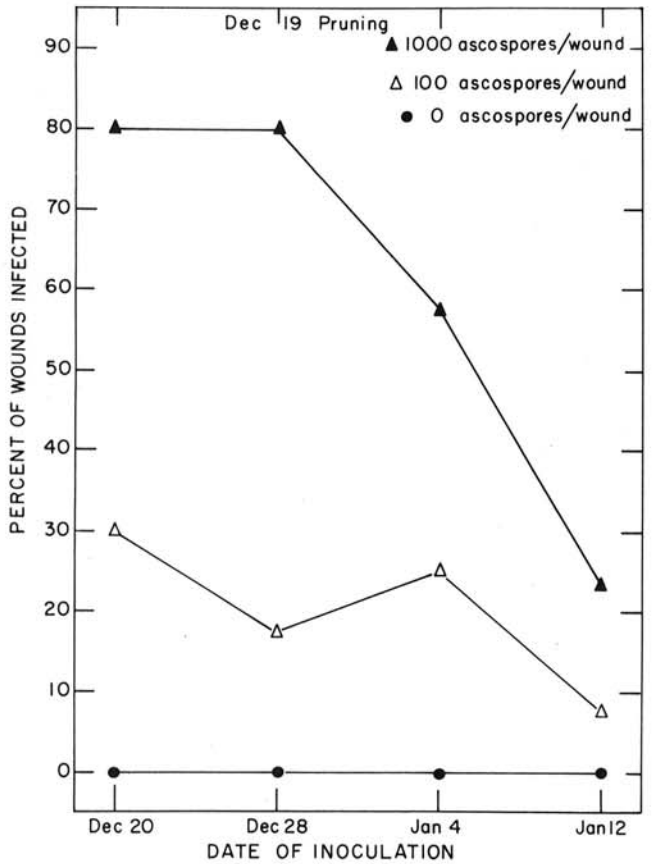

Fig. 3. Percent of grapevine wounds infected with *Eutypa armeniacae* after pruning on 19 December 1978 and inoculating 0, 1, 2, and 3 wk after pruning.

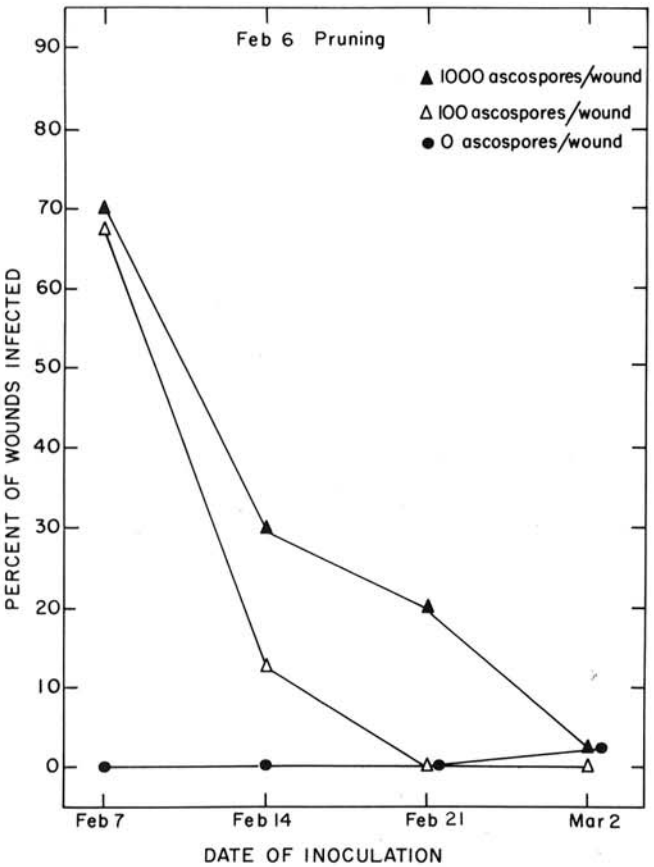

Fig. 4. Percent of wounds infected with *Eutypa armeniacae* after pruning on 6 February 1979 and inoculating at 0, 1, 2, and 3 wk after pruning.

from vines inoculated with distilled water only.

In the spring of 1979, two of the plants that were inoculated 1 yr earlier showed typical Eutypa dieback symptoms (Fig. 2). One of these vines had been inoculated with 20×10^3 ascospores, the other with 50×10^3 ascospores. Cankers were beginning to develop near the original pruning sites. *E. armeniacae* was isolated from canker margins in both cases.

Seasonal differences in susceptibility. Pruning early in the winter resulted in a higher initial rate of infection and in longer susceptibility to infection than pruning later in the winter (Figs. 3–5). When pruning was done in December, wounds were significantly more susceptible to infection for the first 2 wk (three inoculations) than for the third wk ($P<0.01$) after pruning (Fig. 3). Vines pruned in February showed a more rapid decrease in susceptibility, although the initial susceptibility was about the same as for vines pruned in December (Fig. 4). For vines pruned in December or February, wounds were significantly more susceptible on the day of pruning than 1 wk later ($P<0.01$), and wounds inoculated 3 wk after pruning were significantly less susceptible than those inoculated 1 wk after pruning ($P<0.01$). With March pruning, vines were barely susceptible at any time of inoculation including the day of pruning (Fig. 5).

Overall susceptibility was lowest for the March pruning, highest for the December pruning, and intermediate for the February pruning. Chi-square tests showed significant differences among the pruning times for both the 100-ascospore ($\chi^2 = 59.45$) and the 1×10^3-ascospore inoculation levels ($\chi^2 = 24.22$).

Generally the inoculum level of 1×10^3 ascospores per wound produced a higher percentage of infections than 100 ascospores per wound. Statistically significant differences ($P<0.05$) were noted in every case of "moderate" disease susceptibility. Only in cases in which susceptibility was exceedingly high or low were no statistically significant differences seen for the two inoculum levels ($P<0.05$).

Wounds varied from <6 to >11 mm in diameter, but wound size had no significant effect on percentage of wounds infected for a

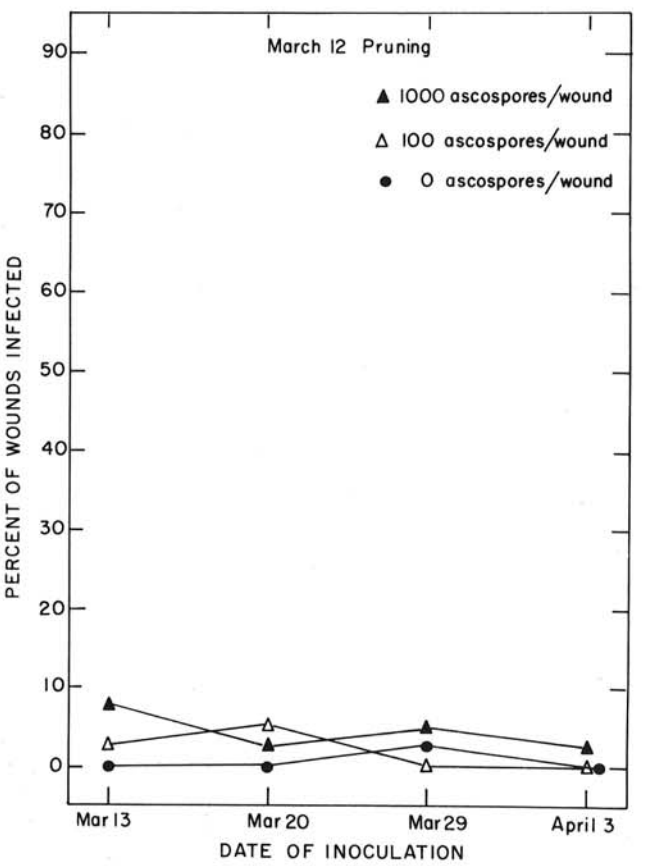

Fig. 5. Percent of wounds infected with *Eutypa armeniacae* after pruning on 12 March 1979 and inoculating at 0, 1, 2, and 3 wk after pruning.

given inoculum level. Using a sign test for independent samples (5), a chi-square statistic showed nonsignificance ($P < 0.05$) for both 100- and 1×10^3-ascospore inoculum levels when numbers of noninfected sites and infected sites were compared with size of the wound.

Relative vertical position of a wound on the vine had no significant effect on the wound's susceptibility to infection ($P < 0.05$).

DISCUSSION

Field inoculation of first-year canes with ascospore suspensions of *E. armeniacae* is useful in determining susceptibility of grapevine to disease. Although 1-yr-old wood may be less susceptible than older wood, its susceptibility to infection after pruning appears to decline at a rate similar to that indicated by the results of Moller and Kasimatis who used older wood (10). Isolation from inoculated sites after an 8-mo incubation period provided relatively rapid results compared to the time required for symptom development and gave a good indication of seasonal variations in wound susceptibility.

The decrease in infection percentage with delayed time of inoculation after pruning may be related to a reduction in the number of sites on the wound surface that can support successful colonization by *E. armeniacae*. These susceptible sites could be unhealed vessels where ascospores can enter. The process of losing susceptibility might be related to vessel healing. This hypothesis is consistent with the February, March, and early January results but is not as consistent with those for late December. However, the reliability of the early December data with a dose of 100 ascospores might be questioned because of the inconsistent pattern of infection frequency.

Susceptibility to infection of first-year grape wood with a given inoculum dose was not correlated with wound size. No correlation would be expected if the number of susceptible sites on a wound is constant per unit area at a given time. Since a constant dose of ascospores was applied in a droplet of uniform size to all wound sizes, the dose per unit area was less for larger wounds. This factor was offset by the presumed greater number of susceptible sites on the surface of a large wound. Thus, no significant differences in infection incidence were attributable to wound size. For naturally disseminated inoculum, large wounds would be expected to receive more inoculum than small wounds and, consequently, have higher probabilities of infection.

Relative position of the wound on the vine apparently does not influence its intrinsic susceptibility. Thus, once ascospores arrive at a pruning wound, the probability of infection is not affected by position on the vine. Differential distribution of infections on vines could reflect differences in inoculum density.

Early winter pruning of grapevines in California undoubtedly provides *E. armeniacae* with the highest probability of successful colonization and infection. Wounds from early winter pruning are highly susceptibile for at least 2 wk after pruning. After 3 wk, wounds can become infected, but the probability is significantly lower.

Susceptibility is initially high for pruning wounds made in midwinter, but then decreases more rapidly than does the susceptibility of early winter wounds. Within 3 wk susceptibility has dropped to nearly zero. Susceptibility of pruning wounds made in late winter or early spring is very low, even on the day of pruning.

Therefore, in areas where ascospore inoculum of *E. armeniacae* is expected to be high, grapevine pruning should be postponed until late winter, because wounds made at this time are least likely to become infected with *E. armeniacae*. The data are similar to the results of Ramos et al (14) for *E. armeniacae* infection of apricots in the reduction of pruning wound susceptibility observed as the breaking of host dormancy approaches. However, apricot pruning wounds appear to be susceptible for a longer period of time than grapevine pruning wounds at each particular time of the dormant season.

LITERATURE CITED

1. Carter, M. V. 1957. *Eutypa armeniacae* Hansf. & Carter sp. nov., an airborne vascular pathogen of *Prunus armeniaca* L. in southern Australia. Aust. J. Bot. 5:21-35.
2. Carter, M. V. 1957. Vines aid spread of apricot gummosis. J. Dep. Agric. S. Aust. 60:482-483.
3. Carter, M. V., and Price, T. V. 1973. *Eutypa armeniacae* associated with vascular disease in grapevine and barberry. Aust. Plant Pathol. Soc. Newsl. 2:27.
4. English, H., and Davis, J. R. 1965. Apricot dieback fungus found on western chokecherry. Plant Dis. Rep. 49:178.
5. Ferguson, G. A. 1966. Statistical Analysis in Psychology and Education. McGraw-Hill, New York. 446 pp.
6. Moller, W. J. 1964. Apricot disease found on garden shrub. J. Dep. Agric. S. Aust. 67:251.
7. Moller, W. J., English, H., and Davis, J. R. 1968. *Eutypa armeniacae* on grape in California. Plant Dis. Rep. 52:751.
8. Moller, W. J., and Kasimatis, A. N. 1975. Newly recognized dying arm disease of grapevines. Calif. Agric. 29(2):10.
9. Moller, W. J., and Kasimatis, A. N. 1978. Dieback of grapevines caused by *Eutypa armeniacae*. Plant Dis. Rep. 62:254-258.
10. Moller, W. J., and Kasimatis, A. N. 1980. Protection of grapevine pruning wounds from Eutypa dieback. Plant Dis. 64:278-280.
11. Moller, W. J., Kasimatis, A. N., and Kissler, J. J. 1974. A dying arm disease of grape in California. Plant Dis. Rep. 58:869-871.
12. Moller, W. J., Ramos, D. E., and Hildreth, W. R. 1971. Apricot pathogen associated with Ceanothus limb dieback in California. Plant Dis. Rep. 55:1006-1008.
13. Pearson, R. C. 1980. Discharge of ascospores of *Eutypa armeniacae* in New York. Plant Dis. 64:171-174.
14. Ramos, D. E., Moller, W. J., and English, H. 1975. Susceptibility of apricot tree pruning wounds to infection by *Eutypa armeniacae*. Phytopathology 65:1359-1364.
15. Samuel, G. 1933. "Gummosis" or "Dieback" in apricot trees. J. Dep. Agric. S. Aust. 36:979-980.
16. Trese, A. T., Burton, C. L. and Ramsdell, D. C. 1980. *Eutypa armeniacae* in Michigan vineyards: Ascospore production, host infection, and fungal growth at low temperatures. Phytopathology 70:788-793.