Susceptibility of Apricot Tree Pruning Wounds to Infection by Eutypa armeniacae

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ABSTRACT

The susceptibility of apricot tree pruning wounds to *Eutypa armeniacae* was evaluated under field and controlled conditions. Pruning wounds made in the fall remained susceptible for at least 42 days, whereas wounds made in the spring became resistant to invasion by the pathogen within 14 days. Wounds made in midwinter were intermediate between those made in the fall and spring. Pruning wounds on young trees held at 20 C became resistant to infection much faster than those maintained in a dormant condition at 3 C. High humidity hastened the development of resistance. Heartwood of large pruning wounds was resistant. A California isolate was more virulent than an Australia isolate of *E. armeniacae*.

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Cytosporina dieback, caused by *Eutypa armeniacae* Hansf. & Carter (imperfect stage, *Cytosporina* sp.), is a serious and economically important disease of apricot *(Prunus armeniaca* L.) trees in Australia (4) and California (10). The symptoms were described in detail by Adam, Grace, and Flentje (2), Wishart (17), and English et al. (12). Pruning wounds are the usual infection sites (1). The fungus first colonizes the xylem, then eventually invades the cambium and bark, resulting in cankers (9). Carter (4) reported that airborne ascospores released during wet weather are the only important inoculum. The ascospores enter the vessels of exposed xylem where germination occurs (5).

Studies of the seasonal abundance of airborne ascospores in South Australia showed a winter period of low ascospore numbers (14). June and July pruning in Australia (corresponding to December and January in the northern hemisphere), when the number of airborne ascospores is low, resulted in reduced natural infection by *E. armeniacae* as compared to spring pruning (13). Carter and Moller (7) showed also that natural infection was lowest following winter pruning, and they observed significantly more infection following fall pruning than following spring pruning.

This paper reports the results of pruning at different times on disease incidence in apricot orchards and presents evidence for a differential resistance to infection in pruning wounds made at different seasons in California. In addition, evidence for differential virulence of *E. armeniacae* isolates from California and Australia is presented.

MATERIALS AND METHODS.—Field experiments were conducted in commercial orchards on bearing apricot trees (cultivar Tilton) in the west side of the San Joaquin Valley. Pruning wounds 1.27 - 1.90 cm in diameter were made just above a lateral shoot or bud with hand shears and were either artifically inoculated or left exposed to natural inoculum.

Two experiments were conducted in controlled environment chambers. Tilton nursery trees, three per 11.4-liter can of pasteurized soil, were used in these studies.

The inoculum was prepared by immersing small pieces of perithecial stromata in water for 0.5 - 1 hours and then suspending it from the lid of a closed petri plate for several hours to collect discharged ascospores. The ascospores were suspended in a small quantity of sterile distilled water and the suspension adjusted to 25 or 100 ascospores per droplet, which were placed on exposed xylem with a Burkard microapplicator. With the exception of fresh wounds, all sites were sprayed with sterile distilled water prior to inoculation. A number of similar-sized drops were also placed on water agar to determine ascospore viability; germination was always near 100%.

Inoculum for single-ascospore inoculations was prepared from a suspension containing one ascospore per 0.25 μliter. Individual 0.25 μliter droplets were placed at each corner of a no. 2 (22 × 22 mm) microscope cover glass and examined at × 25 for the presence of a single spore. The other three drops and spores were then removed. The cover glasses were placed on moistened filter paper in petri plates and an additional 5 μliters of sterile distilled water was added to each spore-bearing droplet. The entire procedure was conducted in a controlled temperature chamber (19 C) with a relative humidity > 80% to prevent evaporation of the droplet.

The single-spore inoculations were made within 6 hours following preparation of the spore suspensions.

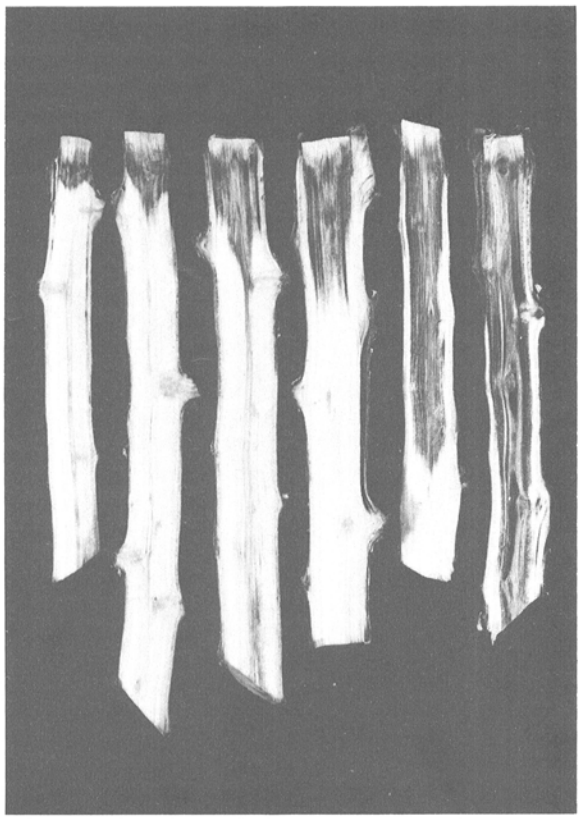

Fig. 1. Split stems of apricot with infected and uninfected pruning wounds showing gradation of internal tissue discoloration. Stem on far left is healthy, whereas stem on far right is infected and was visibly cankered.

The cover glasses were inverted over the fresh pruning wounds with the spore-bearing droplets placed directly between the pith and cambium on the side nearest to a lateral bud or shoot. An additional 20 μliters of sterile distilled water was then applied to each inoculation site to enable the ascospores to be drawn into an exposed vessel.

To assure transfer of the spore from the cover glass to the wound, each cover glass was inverted on water agar plates following inoculation and incubated for 12 hours before being microscopically examined for the presence of germinating ascospores. No *Eutypa* ascospores were found on any of the cover glasses. Spore viability before and after inoculation was near 100%.

After an incubation period of 6-12 months, reisolation from pruning wounds was necessary to determine the incidence of infection because of the long delay in external symptom expression and the difficulty in evaluating internal discoloration as a criterion for infection (Fig. 1). The procedure for reisolation of the pathogen involved: (i) a 1-minute surface sterilization of the split stem with a 1:10 dilution of 5% sodium hypochlorite, (ii) placement of 10 wood chips from the discolored xylem margin on two plates of potato-dextrose agar (PDA), and (iii) visual assessment of the developing fungal colonies after 4-5 days at room temperature. *Cytosporina* colonies are easily recognized and separated from other fungi on PDA by their very distinctive color, morphology, and rate of growth. Reisolation of the pathogen was attempted from all pruning wounds in late summer or fall. A minimum of 6 months was allowed between final pruning or inoculation in each experiment and assessment for infection.

RESULTS.—*Pruning time and the incidence of natural infection by Eutypa armeniacae.*—A differential time-of-pruning experiment was established in an 11-year-old apricot orchard near Tracy, California, where the mean annual rainfall is 228 mm and natural infection is high. A block of 30 trees was divided randomly into six groups of five trees that were pruned on six dates in 1970-71: two in the fall (27 October, 4 November), one in the winter (4 January), and three in the spring (18 February, 4 and 15 March). Isolation of *E. armeniacae* was attempted in the fall of 1971 from twenty pruning wounds on each tree.

Infection of pruning wounds following the October, November, or January pruning dates was significantly higher than that following February or March pruning dates (Table 1). The incidence of infection following January pruning was highest, but does not differ significantly from that following October or November pruning. The high level of infection following January pruning is inconsistent with the results obtained by Carter and Moller (7) in Australia and suggests either greater exposure to airborne ascospores or a longer period of wound susceptibility under conditions prevailing in California.

Our results are different from those reported by English et al. (11) who found increasing canker incidence in apricot trees pruned in summer, autumn, winter, and spring. Their results, however, were obtained in a different climatic area of California and represented cumulative infections over several seasons. Thus, the relationship between time of pruning and exposure to airborne ascospores was an unknown factor.

Seasonal variation in duration of pruning wound susceptibility.—Three experiments involving delayed artificial inoculations after three different pruning dates were carried out on 4-year-old apricot trees near Patterson, California, to determine the possible seasonal effect on duration of wound susceptibility. The three pruning dates were 21 December 1971, 21 September 1972, and 15 March 1973. The wounds were artificially inoculated with 100 ascospores per 5 μliters at 0 (fresh wounds), 14, 28, or 42 days after pruning. Each treatment, including an uninoculated control, was replicated six times in a randomized complete block design with each plot consisting of 20 inoculation sites distributed among five adjacent trees. Thus, there were 120 pruning sites per treatment.

Pruning wounds made in September remained highly susceptible for at least 42 days (Fig. 2), as indicated by the significantly higher incidence of infection (66.5%) of the inoculated wounds over the uninoculated control (23.4%). However, inoculation of 14-day-old wounds after March pruning resulted in the same incidence of infection as observed in noninoculated control wounds (34.7%). The susceptibility of December pruning wounds was about mid-way between the September and March pruning on the basis of the infection percentages for the 14-day-old wounds. Inoculation of 28-day-old pruning wounds made in December did not result in increased infections.

Infection of wounds inoculated on the day of pruning was significantly higher in December than in March. A high infection rate (> 90%) can be expected in fresh wounds made in September on the basis of other experiments (W. J. Moller and D. E. Ramos, *unpublished*).

The incidence of natural infection in September wounds was low, probably because only one light rain (2.3 mm) occurred within 17 days after pruning. Subsequent rains could have occurred during the period of low ascospore production during late fall (15). Unfortunately, spore trapping data for this period are not available to substantiate this explanation. In contrast, the natural infection occurring in the pruning wounds made in March was relatively high, probably because of significant rainfall (14 mm) and high concentrations of airborne ascospores on 19-21 March, 4-6 days after pruning.

Effect of temperature and relative humidity on duration of wound susceptibility.—Experiments were conducted in controlled environment chambers to determine if temperature and relative humidity (RH) influence the rate at which pruning wounds lose their susceptibility to infection by *E. armeniacae*. Light (approximately 5,400 lx at tree height) was provided for 10 hours per day throughout the experiment. Initially the trees (0.79 - 0.95 cm trunk diameter) were pruned to 45 cm above the bud union and held at 3 C from 25 February 1971 until the start of the experiment on 19 March 1971.

On 19 March, 2 April, and 16 April the apricot trees were pruned about 0.63 cm above a lateral bud and about 25 cm from the bud union. The trees pruned on 19 March and 2 April were separated into four groups in which trees with covered or exposed pruning wounds were held at either 3 C (RH = 76% ± 3) or 20 C (RH 60% ± 8). The wounds were covered with polyethylene [0.038-mm (1.5-mil) thickness], held in place by a rubber band. The trees (42 per treatment) were inoculated (100 ascospores each in a 5 μliter droplet per wound) on 16 April. Thus, pruning wounds made on 16 April were inoculated at day 0 and pruning wounds made on 2 April and 19 March were inoculated at 14 and 28 days, respectively, after pruning. Following inoculation, the trees were held in the growth chambers at 19 C for 1 month, after which they were placed in a lathhouse for an additional 5 months before final data were collected and analyzed statistically by the F-test.

Results of this experiment showed there was a significant effect ($P = < 0.05$) of pruning time and a highly significant effect ($P = < 0.01$) of both temperature and wound covering on infection (Fig. 3). There is also a significant interaction ($P = < 0.05$) between temperature and wound covering, which means that changes in one of these variables modifies the effect of the other. Increasing the temperature and RH hastens development of resistance to infection, perhaps as a result of the

TABLE 1. Incidence of natural infection by *Eutypa armeniacae* on apricot trees following six pruning dates[y]

Pruning date (1970-71)	Time from pruning to first exposure to airborne ascospores (days)	Infected wounds[z] (%)
27 October	9	26.8 a
4 November	1	31.6 a
4 January	7	42.4 a
18 February	1	9.3 b
4 March	8	14.4 b
15 March	10	5.4 b

[y]Based on isolation of the pathogen.

[z]Values represent the mean of five replicates. Means followed by different letters are significantly different, $P = 0.05$, as determined by Duncan's multiple range test.

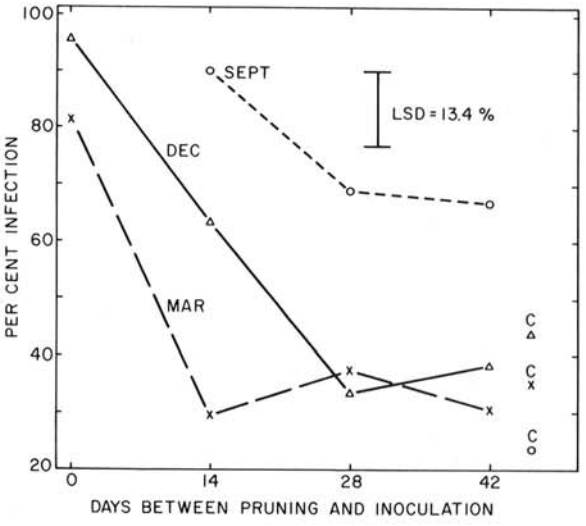

Fig. 2. Relative susceptibility of pruning wounds made in different seasons and artificially inoculated with ascospores of *Eutypa armeniacae*. C, control (natural infection of uninoculated pruning wounds).

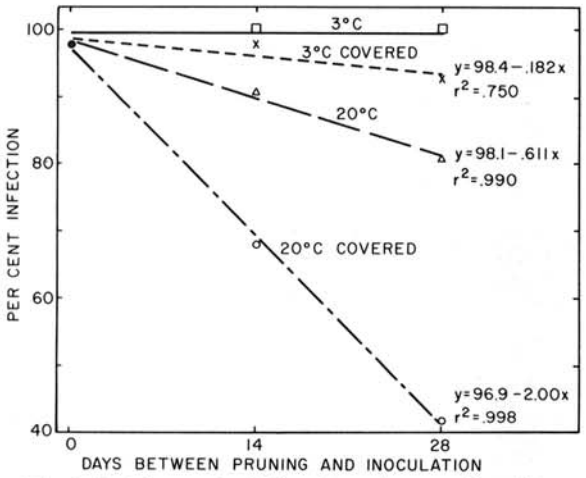

Fig. 3. Relative susceptibility of pruning wounds to *Eutypa armeniacae* under controlled conditions.

accelerated metabolic activity under these conditions. There is a significant ($P = < 0.05$) linear decrease in susceptibility with time in both the covered and uncovered wounds held at 20 C.

The temperatures used in this experiment were selected because they represent the range encountered in apricot orchards in the Patterson area during the transition from winter (January) to spring (March). The mean minimum temperatures in January are usually below 4 C. On the other hand, the mean maximum temperatures in March are around 20 C, which also represents the optimum for both root and shoot growth. In the field, obviously, these conditions are moderated by diurnal fluctuations in temperature.

The polyethylene wrap was used to create a moisture-saturated environment around the pruning wound that would result in a vapor pressure deficit of approximately zero at both temperatures. The condensation of water under the wraps indicated that this was accomplished. The greatest vapor pressure deficit existed on the unwrapped wounds held at 20 C. It appears, therefore, that desiccation of the wounds per se is not responsible for the results obtained. However, at each incubation temperature, wound susceptibility declined more rapidly under the saturated environment.

There was a rapid initiation of growth in trees transferred from 3 C to 20 C. Within 7 days, the buds were obviously growing; there were shoots 5-13 cm long with expanded leaves within 14 days; and the shoots had extended up to 20 cm within 28 days. There was a significant decrease in wound susceptibility with time in the trees held at 20 C, but little change in susceptibility when the trees remained dormant at 3 C.

Site of infection.—An experiment was conducted on 6 October 1971 on a group of 29 mature apricot trees near Patterson to determine which parts of the xylem tissue were susceptible to infection by *E. armeniacae*. Three large (> 3.8 cm diameter) pruning wounds were made on each tree with a saw. Two wounds were immediately inoculated with 25 ascospores each in a 1 μliter droplet applied either to the innermost xylem (heartwood) near the pith or to the outer xylem (sapwood) adjacent to the cambium. The third pruning wound served as an uninoculated control to measure the level of subsequent natural infection. Infection was estimated by external symptoms evident 2 years after inoculation.

In recently differentiated xylem (sapwood), 55.2% of the wounds became infected while only 6.9% of the controls were infected. No infection occurred in the heartwood. The heartwood in these large wounds was characterized by conspicuous brown staining probably due to gums and resins. In contrast, the sapwood was light in color. Apparently, ascospores of *E. armeniacae* are unable to infect heartwood directly through wounds, or the fungus may be unable to grow through the heartwood into the sapwood.

Comparative virulence of California and Australia isolates of Eutypa armeniacae.—Ascospores discharged from pieces of perithecial stromata obtained from Australia were compared with inoculum from the Suisun area of California, using single-ascospore inoculations on fresh wounds. Apricot trees (about 1.27 cm in diameter) were planted in cans (three trees per 11.4-liter can) on 29 February 1972, and placed in a greenhouse. Two of the three trees growing in each can were inoculated on 7 March with a single ascospore of either the California or Australia isolate and the third tree was left as an uninoculated control. Each treatment consisted of 50 trees. Infection frequency was based on positive reisolation of the pathogen from the discolored xylem on 26 June, and canker severity was assessed on the basis of external canker symptoms, principally abnormal

TABLE 2. Comparative virulence of *Eutypa armeniacae* isolates from California and Australia as shown by inoculation of fresh pruning wounds of apricot trees with single ascospores, 7 March 1972[z]

Isolate	Cankers (%)	Total wounds infected (%)	Mean length of tissue discoloration (mm)			
			Infected wounds		Healthy wounds	
			Internal	External	Internal	External
California	46.9 a	71.4 a	77.5	66.1	30.6	12.6
Australia	10.2 b	46.9 b	40.6	24.6	31.4	11.7
Uninoculated check	2.1 b	2.1 c	39.0	13.0	31.9	13.2

[z]Visual canker assessment and attempted reisolation of the pathogen made on 26 June 1972. Total of 49 wounds in the inoculated treatments and 48 wounds in the control. Figures followed by different letters in the same column are significantly different, $P = 0.05$, as determined by χ^2 tests.

gumming. Canker length was determined by measuring the discoloration in the bark tissues, and internal discoloration was measured in the middle of the split stem.

The California isolate of *E. armeniacae* was significantly more virulent than the Australia isolate which was reflected in both infection frequency and canker severity (Table 2). The maximum canker extension, both internal and external, caused by the California isolate exceeded 200 mm, whereas that of the Australia isolate did not surpass 80 mm.

DISCUSSION.—The Tracy-Patterson area of the San Joaquin Valley is a semiarid region in which perithecia, the only known source of inoculum of *E. armeniacae*, have never been observed. However, the high incidence of infection observed after fall and winter pruning may be due to the extended period of wound susceptibility and exposure to airborne ascospores introduced with prevailing air movement from outside sources (15). This is consistent with results obtained by Carter (5) which showed that midwinter wounds remain susceptible to infection for at least 2 months when inoculated with a heavy ascospore suspension under Australian conditions.

In other experiments (15), ten ascospores were as effective as 100 in infecting fresh wounds of apricot trees in California. Thus, the seasonal differences in susceptibility determined with 100 ascospores may predict natural infection since airborne ascospores are usually trapped and presumed to arrive near the infection court in octads.

Seasonal inoculation show that susceptibility of pruning wounds differs markedly at various pruning times. The susceptibility period is longest in the fall, intermediate in winter, and shortest in the spring. The susceptibility periods may be related to the three stages of growth for apricot buds (3). The first growth period occurs during the fall and early winter and is characterized by a very slow rate of growth. The second is a transition period during later winter (January-February) in which the growth rate gradually increases. The third period occurs as bloom approaches and is characterized by a very rapid rate of bud development. The metabolic activity of adjacent woody tissues is thought to follow a similar developmental pattern. In general, the seasonal exhaustion and replenishment of carbohydrate and nutrient reserves reflect the demands of vegetative and reproductive growth. Starch and total carbohydrates accumulate during summer and reach a maximum in autumn. During winter, starch decreases to a minimum while sugars increase to a maximum level. With flowering and resumption of growth in the spring, there is a depletion of starch and sugars. The seasonal differences noted in the susceptibility of pruning wounds may be related to these changes in food reserves in the host tissues.

Wound susceptibility of unsheltered trees in winter in Australia declined within 15 days to a low level, whereas wounds on trees under a rainproof shelter remained susceptible for a much longer period (8). Results obtained from the winter inoculations in Patterson, California resemble more closely those obtained with the sheltered trees in Australia. The mean annual rainfall at the experimental site in Australia was 621 mm compared to 249 mm at Patterson, California. Furthermore, increased air temperature under the plastic tree shelter, although slight, could, under California conditions, cause a significant delay in bud development. This lag in the physiological development of the tissues could be at least partly responsible for the consistently higher wound susceptibility of the sheltered trees. In Australia, susceptibility declined more slowly in early than in late winter on both sheltered and unsheltered trees. This agrees with the results obtained in California.

The rate at which wounds become resistant to infection by *E. armeniacae* is accelerated by moisture. Covering the wounds on trees with a plastic wrap that maintains a moist wound prior to inoculation reduces susceptibility to infection and in Australia tree shelters that exclude rain and allow drying of wounds increase susceptibility. Involvement of other microorganisms in wounds under favorable moisture conditions may render the pruning wounds less suitable for invasion by *E. armeniacae* (6). However, the possibility of moisture enhancing physiological processes during the aging of wounds may also be a factor.

Aging of peach pruning stubs is characterized by the transformation of starch to wound gum (16). Conversion is slower in dry tissues than in tissues maintained in moist conditions. Wound gum formation was more rapid in pruning wounds made during the growing season than in wounds made during the dormant period. *Eutypa armeniacae* can live in wound-gum impregnated heartwood, but failure of symptoms to develop when apricot heartwood was inoculated suggests that the fungus may be unable to directly infect the developed wound-gum region. Heartwood might be too dry, as compared with living wood, or the germinating spores may be unable to utilize heartwood substrates. The development of resistance to infection by pruning wounds might be related to the rate at which stored carbohydrates are transformed to wound gum.

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