Factors Associated with Variation in Susceptibility of Grapevine Pruning Wounds to Infection by *Eutypa lata*

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Accepted for publication 4 November 1994.

ABSTRACT

Munkvold, G. P., and Marois, J. J. 1995. Factors associated with variation in susceptibility of grapevine pruning wounds to infection by *Eutypa lata*. Phytopathology 85:249-256.

Pruning wounds are the only important infection site for *Eutypa lata*, the cause of Eutypa dieback. In 1989–1991, the susceptibility of grapevine pruning wounds inoculated with *E. lata* was significantly affected by date of pruning. Wound susceptibility was highest when vines were pruned early in the dormant season (November or December) and lower when vines were pruned later in the dormant season (January or March). Wound susceptibility declined significantly during the 28 days following pruning, except for fall pruning in one experiment. The decline in susceptibility was highly correlated with an increase in suberin and lignin deposition (r = 0.64–0.92) and with degree-day accumulation (r = 0.83–0.85). The rate of increase of suberin and lignin was also highly correlated with the rate of accumulation of degree-days over 0 C (r = 0.93–0.99). The growth of populations of nonpathogenic microorganisms on the wound surfaces also was dependent on pruning date and was correlated with degree-day accumulation. Microorganism populations increased more rapidly when vines were pruned in early spring than in fall or early winter. The age of wood at the time of pruning did not significantly affect susceptibility. Grape cultivars were significantly different in their susceptibility to infection by *E. lata*, but their relative susceptibility was not always consistent between two field experiments.

Additional keywords: dead arm, *Eutypa armeniacae*, *Vitis vinifera*, wound response.

Eutypa lata (Pers.:Fr.) Tul. & C. Tul. (= *E. armeniacae* Hansf. & M.V. Carter) is a canker pathogen that infects the exposed xylem of wounded woody plants (20). Its primary economic hosts are apricot (*Prunus armeniaca* L.) and grape (*Vitis* spp.), but it infects plants in a wide range of genera (12). During and after rains, *E. lata* perithecia release airborne ascospores, which infect through pruning wounds (40,43). Fruit trees and grapevines are generally pruned during the dormant season; in California, rain is common during this time, so the pruning wounds are potentially exposed to high levels of inoculum.

Eutypa dieback is a chronic disease, especially in grapevines. Infections usually do not produce symptoms for 2–3 yr, while the mycelium colonizes the xylem, cambium, and then phloem tissue (20,31,33). The first noticeable symptoms are stunted shoots with small, chlorotic, cupped leaves. These symptoms are believed to be caused by phytotoxic compounds produced by the fungus, which is confined to the older wood (28,47). The shoot symptoms are associated with a pruning wound canker that typically extends deep into the xylem tissue. The canker eventually girdles the vine, causing death of the cordon or entire vine (11,31,33). Eutypa dieback usually does not appear until a vineyard is at least 8 yr old (11,19). This is partially due to the long incubation period, but other factors may contribute. Older vines require more severe pruning, resulting in larger and more numerous wounds (12). Also, wounds made in older wood have been reported to be more susceptible than those made in 1-yr-old wood (32). Once an epidemic begins, the disease can increase to 90% disease incidence in some 20-yr-old vineyards (19).

Eutypa dieback prevention is based on protection of the pruning wounds with a fungicide (benomyl) and manipulation of the time of pruning (11). Pruning in late winter or early spring is recommended because the numbers of airborne ascospores are low (40,43) and the wounds are believed to be less susceptible (39). Petzoldt et al (39) found that vines pruned in March had very low susceptibility, and the susceptibility of wounds made in February declined more rapidly than that of wounds made in December. The maximum duration of grapevine wound susceptibility is unknown, but wounds that are more than a year old are not susceptible (32). Therefore, infections are likely limited to the same dormant season in which the wounds are made.

Decline in infection of apricot wounds over time has been linked to the presence of large populations of epiphytic microorganisms (10,15,41). In experiments testing materials for pruning wound protection, Carter (10) reported a significant increase in infection by *E. lata* when Bordeaux mix was applied to pruning wounds. He hypothesized that this treatment reduced populations of epiphytic organisms competing with the pathogen (12). Successful biological control of Eutypa dieback has been reported on both grapevine (22,35) and apricot (10).

Another process affecting the susceptibility of pruning wounds is the physiological wound response that occurs in bark and wood (4,7,8,18,24,36,37,45,46). After pruning, the tissue begins to desiccate, and parenchyma cells become necrotic and accumulate higher levels of free and polymerized phenolic compounds (3,24,45). The nature of these compounds depends on the plant species, but accumulation of suberin is common in the wound reaction zones of a range of woody species, including *Vitis* spp. (3,36,37). Xylem vessels can become clogged with tyloses and/or polysaccharide gums (3,21,26,36,37). These tyloses can develop lignified and/or suberized secondary walls (16,21,25,27). The wound response results in the compartmentalization of the wood, which can restrict pathogens within the zone immediately surrounding the wound (46). Wound responses can be identified by histological, biochemical, or molecular methods.

Grapevine cultivars vary in their apparent susceptibility to Eutypa dieback in the field (12,30). In California, cvs. Cabernet Sauvignon, Chenin blanc, and Petite Sirah are believed to be highly susceptible, while cvs. Chardonnay, Merlot, and Tokay are believed to be much less susceptible (unpublished data). It is unknown whether these observed differences are due to physiological susceptibility or to some other factors, such as pruning practices. Some research has indicated that differences in susceptibility are not due to differences in infection; rather, they may

be due to differences in sensitivity to phytotoxic compounds produced by the fungus (28,47).

The objectives of this research were to investigate the relationship between pruning date and susceptibility to Eutypa dieback and to describe the environmental, biological, and physiological factors associated with variation in wound susceptibility. Specifically, we investigated the relationships of cultivar, age of wood, epiphytic populations, temperature, and physiological wound response to wound susceptibility. Preliminary results have been reported (34).

MATERIALS AND METHODS

Inoculum preparation and inoculation. Ascospores of *E. lata* were obtained from perithecia on infected apricot wood collected from Solano Co., California. Pieces of *E. lata* stromata (approximately 4 cm^2) were soaked in sterile water for 1–2 h and then fixed to the lid of a petri dish with petroleum jelly. Ascospores discharged onto water agar in the petri dish and were collected after 6–18 h by removing blocks of agar and placing them in sterile water. The spore concentration was adjusted to 2×10^4 per milliliter, and each inoculated wound received 10^3 ascospores in a 50-μl droplet applied with an automatic pipetter. Wounds were lightly misted with sterile water prior to inoculation.

Effect of pruning date on wound susceptibility. During the dormant season of 1989–90, Thompson Seedless vines in a vineyard near Davis, California, were pruned in the fall, early winter, or early spring and inoculated at weekly intervals. The experimental design was a split plot, with pruning date as the main plot and inoculation date as the subplot. Main plots were replicated in three randomly selected vineyard rows (30 vines per row). Subplots consisted of five pruning wounds on a single vine replicated three or four times within each main plot. The pruning dates were 30 November 1989, 21 January 1990, and 21 March 1991. Inoculation dates were 1, 7, 14, 20, and 28 days after pruning. On each inoculation date, five wounds in each of 10 subplots were inoculated with 10^3 ascospores of *E. lata*. The wounds were on 1-yr-old wood. At the time of each inoculation, 10 randomly selected noninoculated wounds were excised from each main plot (a total of 30 wounds), placed in plastic bags, and transported back to the laboratory in a cooler. Three or four of these excised wounds from each main plot (a total of 10) were immediately frozen at −20 C for later Lignin-thioglycolic acid (LTGA) analysis to detect polymerized phenolics (8,17,23). Ten wounds were assayed for populations of epiphytic microorganisms, and the moisture content of the wood was determined for the remaining 10 wounds.

During 1990–91, the experiment was repeated in a six-row block of Chenin blanc grapevines within a 40-ha vineyard near Courtland, California. The experimental design was a split plot, with pruning date as the main plot and inoculation date as the subplot. The main plots were replicated twice in adjacent rows of 130 vines. Subplots consisted of five pruning wounds on a single vine, replicated 10 times within each main plot. The pruning dates were 14 December 1990, 30 January 1991, and 8 March 1991. Inoculation dates were 1, 7, 14, 21, and 28 days after pruning. On each inoculation date, five wounds in each of 20 subplots were inoculated with 10^3 ascospores of *E. lata*. In each main plot, wounds on 1-yr-old wood were inoculated in five of the subplots, and wounds on 2- or 3-yr-old wood were inoculated in the other five subplots. Wounds (five per main plot on each inoculation date) were sampled for LTGA analysis, epiphytic populations, and moisture content. An equal number of samples was taken from 1-yr-old wood and 2- or 3-yr-old wood. On 21 December 1990, inoculations were not possible due to subfreezing temperatures, so inoculation was delayed until 23 December. In both experiments, a datalogger (Campbell Scientific Micrologger 21X) was placed in the vineyard and the mean, maximum, and minimum temperature and relative humidity were recorded every hour for the duration of the experiment. Precipitation data were obtained from the nearest available weather station.

Since symptom development requires 2–3 yr or more in grapevines (31,33), the results of the inoculations were evaluated by reisolation of the pathogen from the spurs (39). Inoculated spurs were left on the vine until the following dormant season, when they were excised from the vines and brought to the laboratory. The bark was stripped from the spurs, and the portion of the spur extending from the wound surface to the margin of the dead and live wood was retained for reisolation. The spurs were split longitudinally, and each half was then cut into chips approximately 0.5 cm long. The chips were surface-disinfested in 0.5% NaOCl for 3 min, blotted dry on a clean paper towel, and placed in 9-cm petri dishes on a semiselective medium composed of Difco potato-dextrose agar (PDA) amended with streptomycin sulfate at 100 μg/ml, chlortetracycline HCl at 50 μg/ml, and dicloran at 5 μg/ml. The dishes were incubated in the laboratory at ambient temperature (22–25 C) for 1 wk and examined for growth of *E. lata,* which could be readily identified by its colony morphology and growth rate. If any chip from a spur yielded the pathogen, that spur was considered infected.

The number of wounds infected for each subplot (N_i) was determined, and the percentage of wounds infected due to inoculation (P_i) was calculated as $P_i = (100)\,(N_i - N_n)/(5 - N_n)$, where N_n = the mean number of wounds infected in the noninoculated controls. Analysis of covariance was performed on the data, with pruning date as the independent variable and days from pruning to inoculation as the covariate. Linear regression was performed on the effect of days from pruning to inoculation on infection for each pruning date. Slopes were compared by t tests (GLM procedure [44]).

LTGA analysis. The bark was stripped from the frozen specimens and a thin disk of wood (1–2 mm thick, <20 mg) was excised from the wound surface. LTGA analysis was performed as described by Doster and Bostock (17). Nonpolymerized compounds were extracted from the wood disks with three changes of 5 ml of 100% methanol for 24 h each. The specimens were then dried in a vacuum desiccator for 24 h and weighed to the nearest 0.1 mg. Each specimen was then treated with a 10:1 solution of 2 N HCl and thioglycolic acid at 100 C for 4 h. The lignin-thioglycolic acid fraction was then extracted with 0.5 N NaOH for 18 h at 22–24 C. The extract was precipitated by adding concentrated HCl, and the precipitate was collected by centrifugation (10 min at 12,400 rpm). The LTGA pellet was resuspended in 0.5 N NaOH, and the absorbance was measured by spectrophotometer at a wavelength of 280 nm. A standard curve was constructed by suspending an LTGA pellet of known weight and diluting the solution to obtain a linear range of concentrations of LTGA, from 0 to 0.18 mg /ml. The standard curve was $A = (9.95)c$, where A is the percent absorbance at 280 nm and c is the concentration of LTGA in milligrams per milliliter of solution.

Quantification of epiphytic populations. Sampled pruning wounds were brought to the laboratory and immediately stripped of their bark aseptically. A thin disk of wood (1–2 mm thick) was excised from the wound surface. The disks were placed in 50 ml of sterile phosphate buffer (pH 7.0), and placed on a rotary shaker at 150 rpm at 22–24 C for 1 h. An aliquot of 250 μl was then removed from each flask by sterile pipette and spread onto nutrient agar (Difco) in 9-cm petri dishes. The dishes were incubated for 48 h at 22 C, and the total number of colonies was determined for each plate. Colonies included bacteria, yeasts, and filamentous fungi. From these data, the total cfu isolated from each wound was calculated, and the mean cfu per wound per treatment was determined.

Moisture content. A 1- to 2-mm-thick disk was excised from the surface of each wound specimen and weighed to the nearest 0.1 mg. The specimens were oven-dried at 100 C for 2 days and weighed again. The moisture content of each specimen was calculated as $(W_f - W_d)/W_d$, where W_f = fresh weight and W_d = dry weight.

Relative susceptibility of wine grape cultivars. In 1989 and 1991, the relative susceptibility of several wine grape cultivars was investigated at Davis. The experiments were conducted in a vineyard block established in 1965. The block contained 11 vines

of each cultivar planted together in a row, with two cultivars per row. Cultivars included in the experiment were Cabernet Sauvignon, Carignane, Chardonnay, Chenin blanc, French Colombard, Malbec, Petite Sirah, Semillon, and Thompson Seedless. Petite Sirah and Malbec were not included in 1991.

TABLE 1. Linear regression analysis of the effect of wound age at the time of inoculation on infection of grapevine pruning wounds by *Eutypa lata* for three pruning dates during the dormant seasons of 1989–90 and 1990–91[x]

Experiment	Pruning date	Slope	Intercept	R^2
1989–90	30 Nov 1989	−1.27 a	56.0	0.42
	21 Jan 1990	−1.60 a	51.2	0.59
	21 Mar 1990	−1.29 a	34.2	0.59
1990–91	14 Dec 1990	−0.14 c[y]	89.8	0.02[z]
	30 Jan 1991	−2.35 a	83.6	0.70
	08 Mar 1991	−1.36 b	63.3	0.40

[x]Values in a column within the same experiment and followed by different letters are significantly different ($P \leq 0.05$), according to t tests.
[y]Slope was not significantly different from zero ($P = 0.5352$).
[z]Low coefficient of determination was due to inoculation failure on 23 Dec 1990.

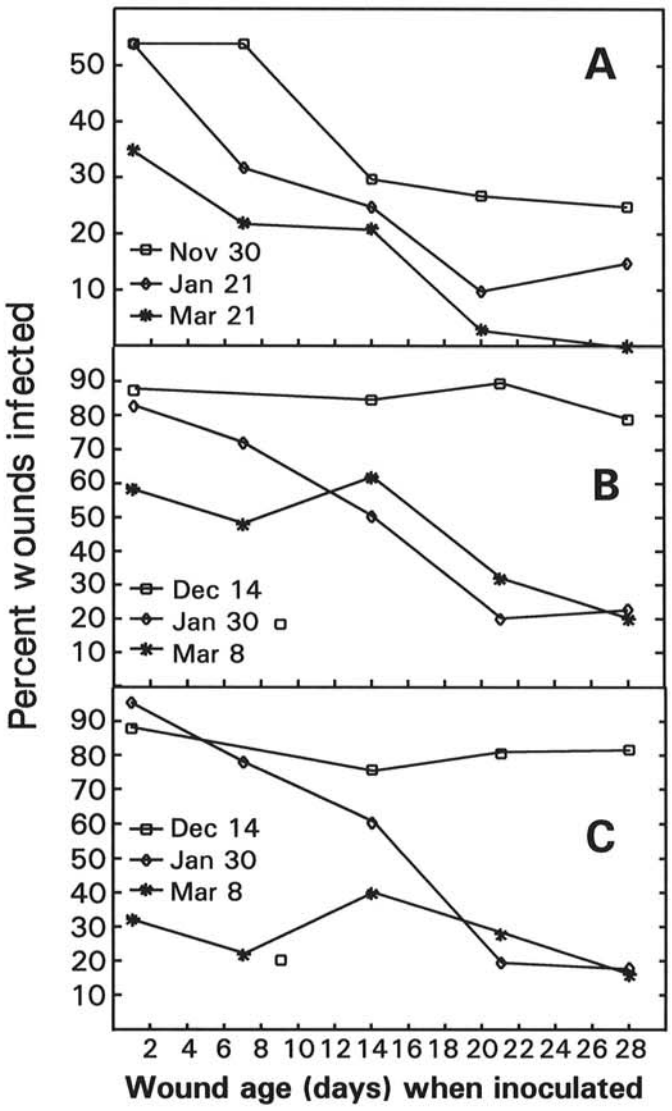

Fig. 1. Mean percent wounds infected after inoculation with 1,000 ascospores of *Eutypa lata* per wound for three pruning dates during the dormant season, **A,** 1989–90 (wounds in 1-yr-old wood), **B,** 1990–91 (wounds in 1-yr-old wood), and **C,** 1990–91 (wounds in 2- or 3-yr-old wood). Data are based on reisolation of the pathogen after about 1 yr. Each point represents the mean of 10 vines.

All vines were head-trained and spur-pruned, except Thompson Seedless, which was cane-pruned. In 1989, the vines were pruned on 11 December, and 25 pruning wounds (five wounds on each of five vines) of each cultivar were inoculated with 10^3 ascospores of *E. lata* on 12 December. At weekly intervals for the next 4 wk, an additional 25 wounds of each cultivar were inoculated. There were insufficient Petite Sirah vines to perform inoculations after 15 days. At each inoculation time, wood specimens were collected from 10 noninoculated wounds per cultivar for LTGA analysis. LTGA analysis was limited to cultivars Cabernet Sauvignon, Chardonnay, Chenin blanc, Petite Sirah, and Thompson Seedless. The 1989 inoculations were left in the field until 25 February 1991, when the spurs were removed and brought to the laboratory for reisolation. For the second experiment, vines were pruned 6 March 1991. The procedure was the same as in the previous experiment, except that inoculations were only performed three times: 1 day after pruning, 8 days after pruning, and 15 days after pruning. The inoculated spurs were collected on 12 January 1992, and infection was determined as described above.

RESULTS

Effect of pruning date on wound susceptibility. For all pruning dates except 14 December 1990, infection of pruning wounds declined significantly ($P \leq 0.05$) as the length of time between pruning and inoculation increased. Initial wound susceptibility varied significantly ($P = 0.0001$) among pruning dates in both years (Table 1, Fig. 1). In 1989–90, there was no significant difference in the rate of decline in susceptibility with wound age among pruning dates ($P = 0.4304$). Over all inoculation times in 1989–90, infection was significantly lower ($P = 0.005$) for the third pruning date but not significantly different ($P = 0.14$) between the other two pruning dates. In 1990–91, susceptibility did not decline significantly with wound age for the first pruning date ($P = 0.5352$), and the rate of decline was significantly greater ($P =$

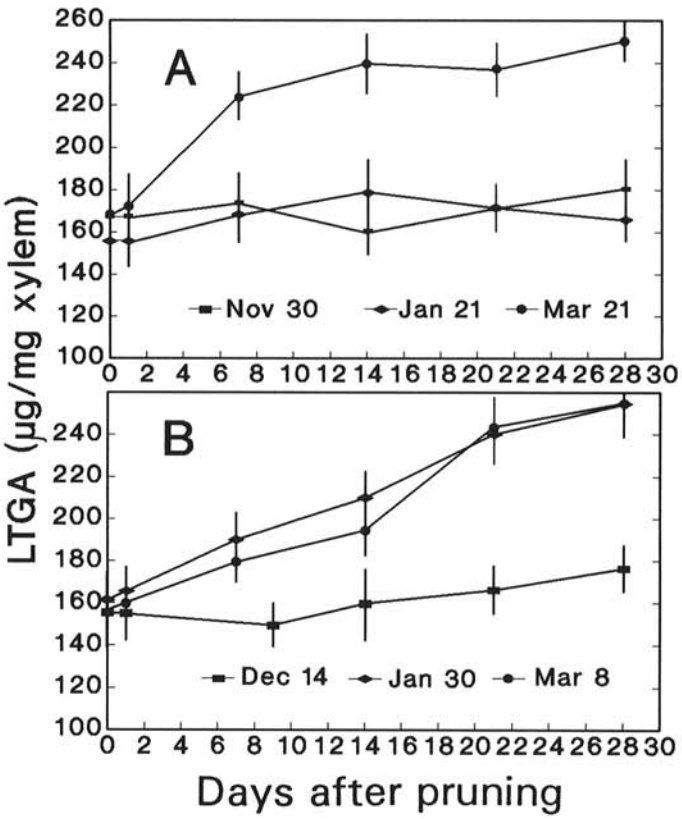

Fig. 2. Mean lignin-thioglycolic acid (LTGA) level in noninoculated wounds for 28 days after pruning for three pruning dates during the dormant season, **A,** 1989–90, and **B,** 1990–91. Wounds were in 1-yr-old wood. Each point represents the mean of 10 wounds. Error bars are one standard deviation above and below the mean.

0.0016) for the second pruning date than for the third pruning date. Overall susceptibility was significantly higher ($P = 0.0026$) for the first pruning date in 1990–91 but not significantly different between the other two pruning dates ($P = 0.1976$). There was no significant effect of wood age at the time of pruning ($P = 0.4256$). In both experiments, the highest levels of infection occurred at the first pruning date. In 1989–90, infection in the noninoculated controls was 2% for the first pruning date and 0% for the other pruning dates. In 1990–91, infection in the noninoculated controls was 24% for the first pruning date, 8% for the second pruning date, and 2% for the third pruning date.

LTGA analysis. Initial LTGA levels in freshly pruned wood were not affected by pruning date. In 1989–90, there was no significant increase in LTGA in wounded tissue ($P > 0.05$) for 28 days after pruning for the first two pruning dates (Fig. 2A). After the third pruning date, LTGA increased during the first 7 days and then remained nearly constant. In 1990–91, LTGA in wounded tissue increased significantly during the 4 wk after the second and third pruning dates ($P = 0.0001$) (Fig. 2B). The rate of increase was similar for the two dates ($P = 0.7897$).

Epiphytic populations. The mean cfu isolated from pruning wounds (expressed as $\log_{10}$ cfu/wound) increased with time after each pruning date (Fig. 3). In both years, populations increased linearly for the first two pruning dates. After the third pruning date, the population increased rapidly during the first day after pruning and then continued to increase slowly (1989–90, Fig. 3A) or remained constant (1990–91, Fig. 3B). In both years, there was a significant effect of pruning date on epiphytic population ($P \leq 0.05$). The composition of the microflora was not quantified, but commonly isolated organisms included *Pseudomonas* spp., *Bacillus* spp., *Aureobasidium pullulans, Cladosporium herbarum, Alternaria* spp., and *Rhodotorula* spp.

Moisture content. The wood at the surface of the wounds decreased in moisture content after pruning (Fig. 4). Initial moisture content was 100–120% for all pruning dates. In 1989–90, the rate of drying was not significantly different among the three pruning dates (Fig. 4A, Table 2). Two weeks after the second pruning date, precipitation occurred on the day the samples were collected, resulting in an increase in moisture content for that day. In 1990–91, the rate of drying was not different for the second two pruning dates, but wounds created on these dates dried more quickly than wounds created on the first pruning date (Fig. 4B, Table 2).

The temperature after pruning varied among pruning dates. Degree-day accumulation after pruning was greater for the 21 March 1990 pruning date (458 after 28 days) compared to the 30 November 1989 (187 after 28 days) and 21 January 1990 (190 after 28 days) pruning dates (Table 2). In contrast, degree-day accumulation after the 30 January 1991 pruning date (359 after

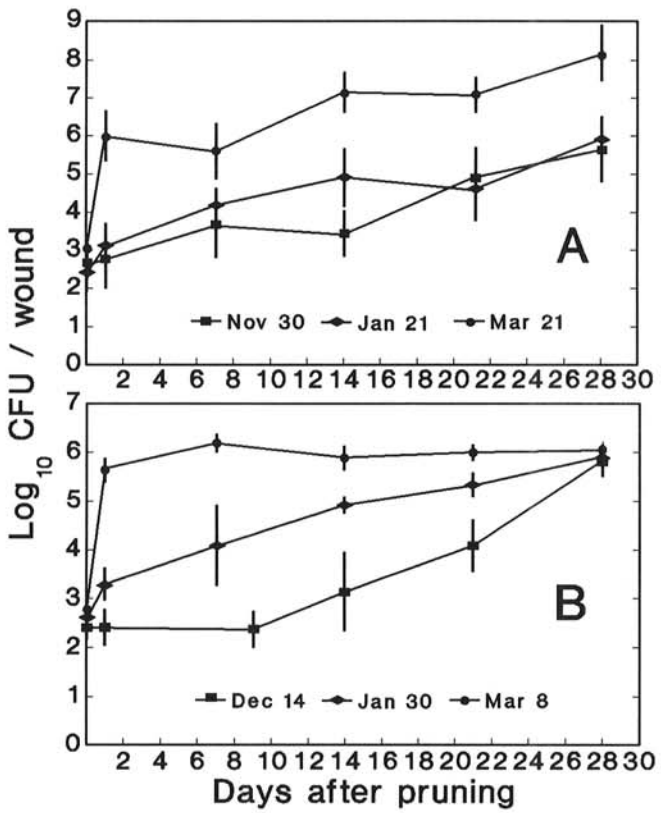

Fig. 3. $\log_{10}$ of the mean cfu isolated from single noninoculated pruning wounds for 28 days after pruning on three different dates during the dormant season, **A**, 1989–90, and **B**, 1990–91. Each point represents the mean of 10 wounds. Error bars are one standard deviation above and below the mean.

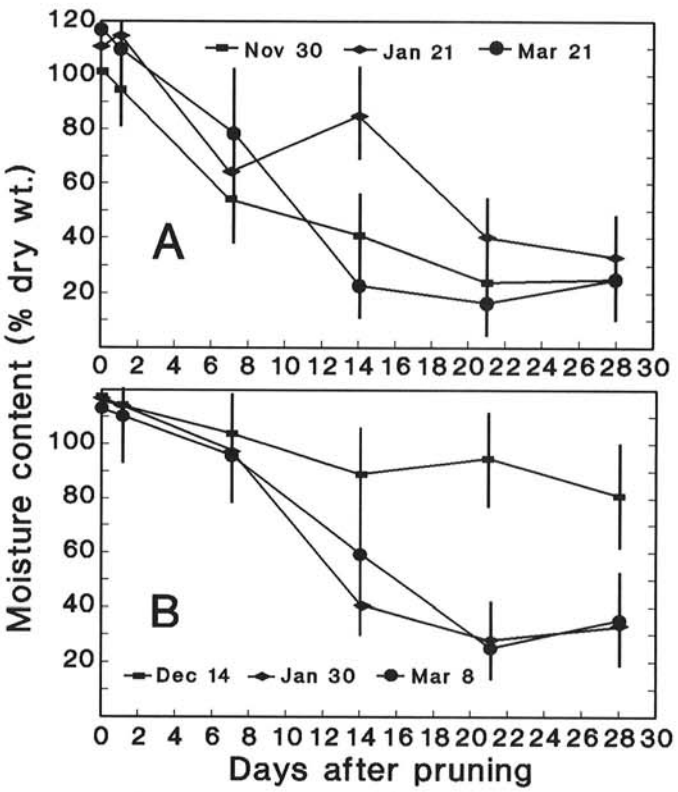

Fig. 4. Mean moisture content of wood at the site of pruning in noninoculated wounds for 28 days after pruning for three pruning dates during the dormant season, **A**, 1989–90, and **B**, 1990–91. Each point represents the mean of 10 wounds. Error bars are one standard deviation above and below the mean.

TABLE 2. Slope coefficients for linear regression analysis of the change over time in variables associated with decline in susceptibility of grapevine pruning wounds calculated for three pruning dates during the dormant seasons of 1989–90 and 1990–91[x]

Experiment Pruning date	Degree-days[y]	Epiphytic population[z]	Lignin-thioglycolic acid	Moisture content
1989–90				
30 Nov 1989	0.38	0.107	0.38 b	−2.5 a
21 Jan 1990	0.83	0.088	0.36 b	−2.7 a
21 Mar 1990	15.92	0.086	2.46 a	−3.4 a
1990–91				
14 Dec 1990	4.89	0.105	0.87 b	−1.0 b
30 Jan 1991	12.94	0.116	3.47 a	−3.3 a
08 Mar 1991	10.83	0.091	3.84 a	−3.2 a

[x] Variables were measured weekly for 28 days after pruning. Values in a column within the same experiment and followed by different letters are significantly different ($P \leq 0.05$). Slopes were compared by covariance analysis and t tests.
[y] Degree-days above 0 C.
[z] No significant differences in column ($P > 0.05$).

TABLE 3. Correlation coefficients for relationships among wound age, accumulation of degree-days above 0 C, epiphytic populations on the wound surface, lignin/suberin accumulation, wood moisture content, and infection of grapevine pruning wounds after inoculation with ascospores of *Eutypa lata*

Experiment	Degree-days	Epiphytic population[v]	Lignin-TGA[w]	Moisture content[x]	Infection
1989–90					
Wound age[y]	0.7907**[z]	0.5852*	0.3286	−0.8438**	−0.7789**
Degree-days		0.8174**	0.7776**	−0.7726**	−0.8334**
Epiphytic population			0.8228**	−0.5366*	−0.8093**
Lignin-TGA				−0.4542	−0.6388*
Moisture content					0.6766**
1990–91					
Wound age	0.8446**	0.7388**	0.7070**	−0.7441**	−0.5198*
Degree-days		0.7707**	0.9530**	−0.9154**	−0.8478**
Epiphytic population			0.7327**	−0.7411**	−0.6819**
Lignin-TGA				−0.9375**	−0.9161**
Moisture content					0.8437**

[v] Measured as $\log_{10}$ of cfu isolated from pruning wounds at the time of inoculation.
[w] Lignin-TGA = lignin-thioglycolic acid accumulation (a measure of lignin and suberin response to wounding).
[x] Moisture content of the wounded wood at the time of inoculation measured as percent dry weight of wood.
[y] Wound age in days at the time of inoculation.
[z] * = Correlation significant at $P \leq 0.05$; ** = correlation significant at $P \leq 0.01$.

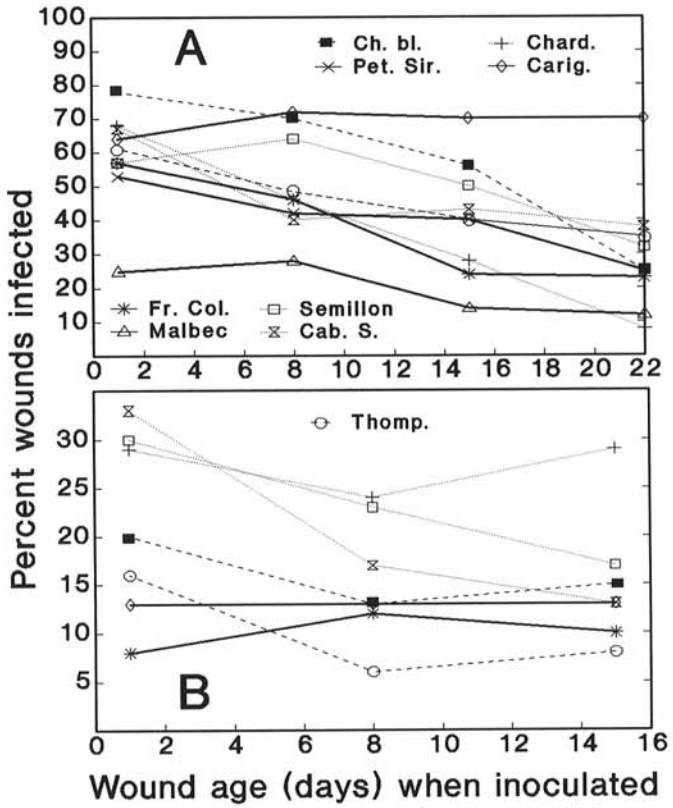

Fig. 5. Mean percent wounds infected after inoculation with 1,000 ascospores of *E. lata* per wound for different grape cultivars pruned on **A**, 12 December 1989, or **B**, 6 March 1991. Wounds were in 1-yr-old wood. Data are based on reisolation of the pathogen after about 1 yr. Each point represents the mean of five vines. Abbreviations are: Ch. bl. = Chenin blanc, Chard. = Chardonnay, Pet. Sir. = Petite Sirah, Carig. = Carignane, Fr. Col. = French Colombard, Cab. S. = Cabernet Sauvignon, and Thomp. = Thompson Seedless.

28 days) was slightly higher than after the 8 March 1991 pruning (318 after 28 days), while it was much lower for the 14 December 1990 pruning (145 after 28 days).

During both years, there were highly significant correlations among degree-day accumulation, days between pruning and inoculation, LTGA accumulation, epiphytic populations, moisture content, and infection level (Table 3).

Relative susceptibility of cultivars. In 1989, infection declined significantly ($P \leq 0.05$) as the length of time between pruning and inoculation increased for all cultivars except Carignane and Malbec (Fig. 5A, Table 4). Over all inoculation times, infection varied significantly among cultivars. Carignane and Chenin blanc had the highest mean infection levels, and Malbec had the lowest. Chardonnay had the greatest rate of decline in susceptibility.

TABLE 4. Linear regression coefficients for the effect wound age at the time of inoculation on infection of grapevine pruning wounds by ascospores of *Eutypa lata* for nine winegrape cultivars inoculated in 1989 and 1990

Experiment Cultivar	Slope	Intercept	R^2	Overall mean[z]
1989				
Carignane	0.23	66.4	0.02	69.0
Chenin blanc	−2.17	80.0	0.57	55.0
Semillon	−1.23	64.6	0.22	50.5
Cabernet Sauvignon	−1.31	62.1	0.28	47.0
Thompson Seedless	−1.14	59.1	0.28	46.0
Petite Sirah	−1.31	55.1	0.34	40.0
French Colombard	−1.68	56.9	0.40	37.5
Chardonnay	−2.82	70.0	0.68	37.5
Malbec	−0.71	27.7	0.11	19.5
1991				
Chardonnay	0.00	26.7	0.00	26.7
Semillon	−0.93	31.3	0.16	23.3
Cabernet Sauvignon	−1.71	35.0	0.33	21.3
Chenin blanc	−0.29	18.3	0.02	16.0
Carignane	0.00	13.0	0.00	13.0
French Colombard	0.14	8.9	0.01	9.7
Thompson Seedless	−0.57	14.6	0.07	9.7

[z] Mean percentage of wounds infected for all inoculation dates.

Incidence of infection was lower in 1991 than in 1989 (Fig 5B, Table 4). Infection did not decrease significantly ($P > 0.05$) as the length of time between pruning and inoculation increased for any cultivar except Cabernet Sauvignon ($P = 0.0075$). Over all inoculation times, infection varied significantly among cultivars ($P = 0.0031$). However, the ranking of cultivars with respect to intercept, slope, or overall mean was not consistent between the two experiments (Table 4).

In 1989, LTGA levels varied significantly among cultivars ($P = 0.0327$) (Fig. 6A). There was no significant increase in LTGA over time for any cultivar ($P > 0.05$). In 1991, there was no significant difference in overall LTGA among cultivars ($P = 0.3791$) (Fig. 6B). LTGA increased significantly over time for all cultivars.

DISCUSSION

Wound age and date of pruning have been shown to affect susceptibility to canker pathogens in almond, apple, apricot, grape, and peach (4,6,13,14,17,18,26,39,42). The results of this research confirmed the importance of late pruning for reduction

of grapevine pruning wound infections by *E. lata*. However, infection levels obtained in this study indicated that susceptibility of pruning wounds may remain high for a much longer duration than previously believed. Previous estimates of duration of susceptibility were based on a single inoculation experiment (39), which indicated very low susceptibility for March pruning and a 75–100% reduction in infection 4 wk after pruning in February or December. In our study, infection levels were much higher than previously reported for March pruning, and the reduction in susceptibility over time was variable, depending on environmental conditions. Susceptibility did not decline at all for 28 days after the 14 December 1990 pruning (Fig. 1B and C).

LTGA analysis is a quantitative biochemical method to determine the amounts of polymerized phenolic compounds (lignin and the phenolic component of suberin) in plant tissue (8,17,23). Temperature is known to affect wound response and, particularly, LTGA accumulation in wounded woody plants (2,18). Low temperatures after wounding have also been associated with longer durations of susceptibility to wound pathogens of woody plants (2,18). During the dormant season in California, temperatures during the weeks following pruning will typically be lowest if pruning is performed in December and highest if pruning is performed in March. However, the results of this study demonstrate that this can vary. Degree-day accumulation was lowest for the 4-wk period following the January pruning date in 1989–90; yet in 1990–91, it was highest following the January pruning date. This correlated well with the LTGA and infection results (Figs. 1 and 2, Tables 1–3). When degree-day accumulation was low, LTGA accumulation was low and susceptibility declined slowly if at all. LTGA level and infection frequency were better correlated with degree-day accumulation than with wound age at the time of inoculation (Table 3). These results indicate that the best pruning time for disease prevention depends on temperature after pruning, rather than simply on pruning date. In other pathosystems involving wound pathogens, degree-day accumulation has been used to predict wound susceptibility (2). Such a system may be applicable to Eutypa dieback, although other factors also influence susceptibility. For example, infection rates for inoculation one day after pruning were lower when vines were pruned in March compared to other pruning dates. Wounds made at this time exude large volumes of fluid from the xylem, up to 11.9 ml/h (1) during the first days after pruning. This may inhibit the establishment of *E. lata* ascospores in the xylem by physical flushing.

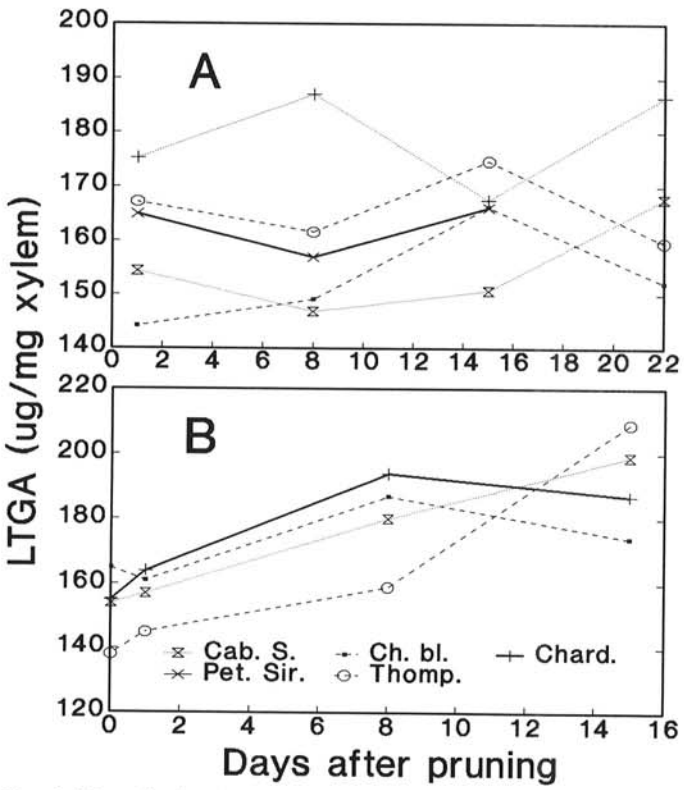

Fig. 6. Mean lignin-thioglycolic acid (LTGA) level in noninoculated wounds for different grape cultivars after pruning on **A**, 12 December 1989, or **B**, 6 March 1991. Each point represents the mean of 10 wounds. Abbreviations are: Ch. bl. = Chenin blanc, Chard. = Chardonnay, Pet. Sir. = Petite Sirah, Cab. S. = Cabernet Sauvignon, and Thomp. = Thompson Seedless.

Temperature also affects the growth of nonpathogenic colonizers of the pruning wounds, which may reduce the ability of *E. lata* to infect, due to competition or other types of antagonism. The increase in epiphytic populations on the wounds was much more pronounced for the March pruning dates (Fig. 3). While this was positively correlated with higher temperatures (Table 3), temperature alone does not explain the high populations detected only 1 day after pruning. One explanation for this could be the profuse xylem exudation that occurs when vines are pruned near the end of the dormant season. This exudate contains carbohydrates, amino acids, and organic acids that may promote rapid growth of the wound microflora (1,35) that compete with the pathogen. Increased competition may contribute to low infection levels for the early inoculations after March pruning dates. However, nutrients in the xylem fluid may stimulate the growth of *E. lata* as well. Therefore, the net effect of xylem exudation is unknown.

The drying of wood tissue is associated with wound response (45), but the critical moisture content required to inhibit infection by *E. lata* is unknown. Drying of the tissue was correlated with temperature, LTGA, and a loss of susceptibility in this study (Table 3).

Inoculations of different grapevine cultivars in this study indicated significant differences in susceptibility, but the relative susceptibility was not consistent between the two experiments. In 1991, infection levels were very low, and differences in susceptibility were small. The ranking of cultivars based on these results is probably not reliable. The 1989 results are more consistent with field observations. For example, Chardonnay had the steepest decline in infection and one of the lowest overall means. Clearly, a more reliable means of evaluating the susceptibility of cultivars is needed. If relative susceptibility depends on toxin sensitivity rather than on resistance to infection, it would not be detected by our infection assay.

Differential susceptibility of cultivars to wound pathogens can sometimes be related to wound response characteristics (4,6). In the 1989 experiment, significant differences in LTGA were detected among the cultivars. The cultivar with the highest LTGA level also was among the least susceptible (Chardonnay). However, LTGA did not increase measurably after pruning in this experiment, and the ranking of cultivars was quite different in 1991 with respect to LTGA and susceptibility (Table 4, Figs. 5 and 6). The 1989 experiment was conducted in December, and the 1991 experiment was conducted in March. This may have caused inconsistent results, due to differences in lignin and suberin accumulation rates between the two experiments (Fig. 6). Similarly, Biggs and Miles (6) found that suberin accumulation in May and June was significantly correlated with relative wound susceptibility to *Leucostoma* spp. among peach cultivars, but this correlation was not significant in September or October. Since the LTGA method measures both lignin and suberin, it is not as specific as histochemical methods used in the study by Biggs and Miles (6).

Observations of cankers have led to the belief that wounds in older wood are more susceptible to Eutypa dieback than wounds in first-year wood (11,12). This may be due partially to wound size, but in an inoculation study, Moller and Kasimatis (32) reported a significantly higher level of susceptibility in fresh wounds on 2-yr-old wood compared to 1-yr-old wood. This was based on a total of 40 inoculated wounds. In the present study, there was no significant difference in infection of 1-yr-old and 2- or 3-yr-old wood (1,500 wounds) inoculated with the same inoculum dose (Fig. 1B and C). In fact, for some inoculations, the percentage of infected wounds was lower in the older wood.

Other researchers have also failed to detect any difference in wound susceptibility according to age of wood (48). This result has important implications for disease management. Based on the assumption that wounds in older wood are more susceptible, some authors have recommended that pruning wound protection need not be applied to wounds in annual wood (11,12). In fact, these wounds appear to be equally susceptible, and wound protection for all pruning wounds should be recommended.

The inoculum dose used in this study was higher than can be expected under natural conditions (11,43). However, this should not have biased the results with respect to relative susceptibility of wounds, and the results are comparable to other studies using the same inoculum dose (39).

The direct effects of environmental conditions on the ability of *E. lata* to infect pruning wounds were not studied here, although these effects no doubt influenced our results. The optimum temperature for germination and growth has been reported to be 22–25 C, and relative humidity (RH) of at least 90% is required for germination (9). The minimum RH must be maintained for a longer period if temperatures are below 15 C (9). The temperature optimum does not relate well to the results in Figure 1. During the course of the experiments, temperatures were consistently below 20 C for the first two pruning dates and did not reach 22 C until 20 March 1990 and 13 February 1991, so the near optimum temperatures coincided with low infection rates. RH during the 48 h following inoculation was similar for the first two pruning dates in both experiments, with minima of 22–60% and maxima of 85–96%. RH minima were lower for the third pruning date (15–48%). Low RH may have reduced infection for the third pruning date. Precipitation was below normal both years; only traces fell during the 1989–90 experiment. Rain occurred several times following inoculation in the 1990–91 experiment, primarily following the second two pruning dates, when infection levels were lower. These data suggest that wound susceptibility had a stronger effect on infection than did the environment–inoculum interaction, except for the inoculation on 23 December 1990, when subfreezing temperatures prevented infection.

The mechanisms that influence the decline in susceptibility of pruned wood may include host wound response and competition with epiphytic populations. Through these mechanisms, temperature strongly affects the susceptibility of pruning wounds. Knowledge regarding the relationship between temperature and wound response, coupled with studies regarding the direct influence of these and other factors on the pathogen, could lead to accurate prediction of infection levels for specific pruning dates. Any predictive model also must consider the periodicity and magnitude of inoculum dose in the air, which may have a strong effect on seasonal patterns of infection, independent of wound susceptibility. Previous studies have shown that seasonal differences in inoculum concentration occur, but these studies have not included infection data (29,38,40,43,48). Studies on seasonal differences in infection have relied on artificial inoculation (13,14,39,42).

An understanding of wound response mechanisms also is important in developing strategies to reduce wound susceptibility by promoting wound response. Potentially, this can be accomplished through chemical (7) or cultural (5) practices.

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