The Effects of Fungicides on *Eutypa lata* Germination, Growth, and Infection of Grapevines

G. P. MUNKVOLD and J. J. MAROIS, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

Munkvold, G. P., and Marois, J. J. 1993. The effects of fungicides on *Eutypa lata* germination, growth, and infection of grapevines. Plant Dis. 77:50-55.

The fungicides benomyl, fenarimol, flusilazole, iprodione, myclobutanil, triadimefon, and vinclozolin were evaluated for their efficacy in vitro against *Eutypa lata*. For inhibition of ascospore germination, flusilazole was most effective, with an EC_{50} value of 0.06 μg a.i./ml. Fenarimol and myclobutanil were somewhat less effective, with EC_{50} values near 1 μg/ml. Benomyl, iprodione, triadimefon, and vinclozolin did not effectively inhibit germination. Flusilazole was also the most effective fungicide for inhibition of mycelial growth. Benomyl, fenarimol, flusilazole, and myclobutanil had EC_{50} values less than 1.0 μg/ml for mycelial growth, whereas iprodione, triadimefon, and vinclozolin had EC_{50} values ranging from 1.49 to 6.27 μg/ml. Benomyl, fenarimol, flusilazole, myclobutanil, and triadimefon were tested in the field for their ability to prevent infection of artificially inoculated grapevine pruning wounds. In four field experiments, benomyl and flusilazole were consistently the most effective chemicals, reducing the percentage of infected wounds by over 90% in one experiment. When inoculations were performed 14 days after fungicide application, all fungicides were less effective than when inoculations were performed after 1 or 2 days, with the exception of flusilazole. Fenarimol, myclobutanil, and triadimefon were less effective when precipitation occurred immediately after fungicide application. A pneumatic sprayer–pruning shear was as effective as application of fungicides by paintbrush.

Additional keywords: deadarm, dieback

Eutypa dieback of grapevines (*Vitis vinifera* L.), caused by the fungus *Eutypa lata* (Pers.:Fr.) Tul. & C. Tul. (syn. *E. armeniacae* Hansf. & M. V. Carter), is a serious disease in northern California and occurs in other grape-growing areas in the United States, Australia, and Europe (4). The same fungus also causes dieback of apricots (*Prunus armeniaca* L.), and isolates from grape and apricot are pathogenic to both hosts (2,5,25). The disease is spread by airborne ascospores, which infect the open vessel elements in the xylem of pruning wounds. The fungus slowly colonizes the xylem, cambium, and phloem, resulting in a canker in 2–4 yr (11,15,17). Shoots arising from the wood near the canker are stunted and distorted and do not produce fruit. If the infected portion of the vine is not removed, the canker spreads to the trunk and kills the vine (15,17). Infection and sporulation are favored by high rainfall or sprinkler irrigation (13). Eutypa dieback is most severe in vineyards over 15-yr old (10,17). Currently, management of Eutypa dieback relies on timing of pruning and wound protection with benomyl. Vines pruned late in the dormant season are believed to be less susceptible than those pruned earlier (23), but late pruning is not practical for many growers. Different pruning and training methods may reduce losses by this disease, but this has not been thoroughly researched.

Pruning wound protection with benomyl and/or wound sealants is practiced by most growers where the disease is severe. Moller et al (18) found that benomyl was more effective than wound sealants applied to the pruning wounds of apricots. Other researchers have confirmed that benomyl is effective in preventing infections of both apricot and grape (12,14,16,21). However, benomyl is not generally effective when applied by air or ground sprayers (9,18). The fungicide is most effective when applied directly to each wound at a high concentration (1.25% a.i. or 12,500 μg a.i./ml [all subsequent references to fungicide concentrations are in units of a.i.]) by paint brush or hand sprayer (18). Benomyl has been registered in the United States for Eutypa dieback prevention since 1976.

Despite the routine use of benomyl, Eutypa dieback of grapevines has become more severe in northern California during the past 20 yr. One possible explanation for this is that certain popular wine grape cultivars are now well established, and many vineyards in the state are now 15-yr old or older. These older vines are more seriously affected by Eutypa dieback. In addition, pruning wounds may be susceptible for more than a month after pruning (19,23), and there is some question whether a single benomyl application can protect the wounds for that duration (18,24). Infections occur during the winter, when rainfall is common, and the fungicide may not persist under these conditions.

The effectiveness of benomyl on growth and germination of *E. lata* in vitro was examined by Carter and Price (8). Germination was completely inhibited at 20 μg/ml, and mycelial growth was completely inhibited at 0.3 μg/ml. The effects of the other fungicides used in the present study have not previously been reported.

The objective of this research was to compare the efficacy of benomyl with that of several newer fungicides against *E. lata* in vitro and then to determine whether other fungicides were more effective at preventing infection of grapevines by *E. lata*. In addition, an alternative application method, a pneumatic sprayer–pruning shear, was evaluated. A portion of these results has been reported previously (20).

MATERIALS AND METHODS

Fungicides evaluated. Seven fungicides were evaluated in the laboratory, and five of the seven were tested in the field. These were benomyl (Benlate 50 WP), fenarimol (Rubigan 12% EC), flusilazole (Nustar 20 DF), iprodione (Rovral 50 WP), myclobutanil (Rally 40 WP), triadimefon (Bayleton 50 WP), and vinclozolin (Ronilan 50 DF). Benomyl is the only fungicide registered in the United States for pruning wound protection. Fenarimol, iprodione, myclobutanil, and triadimefon are registered for use on grapevines for other diseases. Flusilazole and vinclozolin are not registered for use on grapevines in the United States. Fungicides used in the field were benomyl, fenarimol, flusilazole, myclobutanil, and triadimefon.

In vitro experiments. All seven fungicides were evaluated for their ability to inhibit radial growth and ascospore germination. Three isolates of *E. lata* from California were used for radial growth studies. Isolate GC13 was isolated from a cankered Cabernet franc vine from Sonoma County, isolate GC16 was isolated from a cankered French Colombard vine from Merced County, and isolate GA17 was an isolate grown from ascospores from a Sauvignon blanc vine from Napa County. The isolates were stored on potato-dextrose agar (PDA) slants at 4 C and transferred to PDA in petri dishes for propagation. They were then incubated under continuous light at room temperature for 1 wk, at which time there was sufficient growth

Accepted for publication 17 September 1992.

for transfer to fungicide-amended media. The fungicides were suspended in sterile distilled water and added to molten PDA at approximately 50 C in the proper amounts to achieve final concentrations of 0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 μg/ml. One 8-mm diameter plug of mycelium from the margin of an actively growing culture was transferred to the center of each dish. There were seven replicates of each fungicide concentration, and the experiment was performed three times. The dishes were incubated for 1 wk in a dark, 22 C incubator, and then the diameter of the colony in each culture dish was measured to the nearest millimeter. The diameter of the original mycelial plug was subtracted prior to analysis of the data.

Ascospores were obtained from perithecial stromata on apricot wood from Solano County, California. Apricot was used as the source because sufficient amounts of spores from grape were not always available. The stromata were chipped into pieces about 4 cm^2, soaked for 1 or 2 hr in sterile distilled water, and then fixed to the lid of a plastic petri dish with petroleum jelly. The lids were placed on the dishes, which contained water agar, and sealed with Parafilm. After 6–18 hr, a sufficient number of ascospores had usually discharged onto the water agar. Aggregations of ascospores were removed by aseptically excising a block of the agar. The block was then placed into sterile distilled water and agitated. The concentration of the spore suspension was adjusted to about 10^5/ml. Media were prepared as described above, with final fungicide concentrations of 0, 0.1, 0.5, 1.0, 5.0, and 10.0 μg/ml. A 200-μl aliqot of the spore suspension was pipetted onto each dish and spread into a thin layer. There were two dishes of each fungicide concentration, and the experiment was performed three times. The dishes were incubated for 48 hr at 22 C in the dark, and then percent germination was determined. On each dish, 400 spores were counted and the percentage of these that had germinated was calculated. Spores were considered germinated if the germ tube was as long as the spore.

EC_{50} values (the concentration required to reduce radial growth or germination by 50%) were calculated by a regression of the probit of the percent inhibition for each fungicide concentration over the $\log_{10}$ of the fungicide concentration. Two-way analysis of variance was performed on the EC_{50} estimates for radial growth from the three experiments, and the means were separated by Duncan's multiple range test (DMRT) (26). One-way analysis of variance was performed on the EC_{50} estimates for germination from the three experiments.

Dishes of PDA amended with 10 μg/ml of benomyl were routinely inoculated with suspensions of ascospores from different sources in order to screen for resistant isolates. Ascospore suspensions were prepared as above, and dishes were incubated on the lab bench for 2 wk, after which they were inspected for growth of *E. lata* colonies. Any isolate growing on this medium was considered resistant to benomyl. Several million ascospores from two apricot and four cherry orchards and two vineyards were screened in this manner during the course of the study.

Field experiments. In both 1990 and 1991, two field experiments were conducted: one during the spring of 1990 (90S), one during the fall of 1990 (90F), and two during the winter of 1991 (91WA and 91WB). Experiments 90S, 90F, and 91WB were conducted in different sections of the same cordon-trained Chenin blanc vineyard, located in Sacramento County, California, planted in 1969. Experiment 91WA was conducted in a cordon-trained Chenin blanc vineyard in Merced County, California, planted in 1983.

In each experiment, fungicides were applied to all pruning wounds on the treated vines, but only five wounds (spurs) per vine were selected for inoculation. Wounds were on 1-yr-old wood, approximately 1–2 cm in diameter. Inoculations were performed by lightly misting each wound with sterile distilled water and applying 1,000 ascospores of *E. lata* in a 50-μl droplet of sterile distilled water. Noninoculated controls were misted only. The fungicides were all applied at 12,500 μg/ml with a paint brush. Tap water only was applied to the nontreated controls.

Experiment 90S was in a randomized complete-block design in two blocks of 60 vines each. There were six treatments per block, with 10 individual vines randomly assigned to each treatment. Treatments were four fungicides (benomyl, fenarimol, myclobutanil, and triadimefon), a nontreated control, and a noninoculated control. Vines were pruned on 3 March 1990, and the fungicide treatments were applied within 2 hr after pruning. Inoculations were performed after 24 hr.

Experiment 90F was a 6 × 2 factorial with a noninoculated control in a completely randomized design. The first factor was fungicide treatment. This experiment included five fungicides (benomyl, fenarimol, flusilazole, myclobutanil, and triadimefon) and a nontreated control. The second factor was inoculation date. For each fungicide treatment, 10 vines were inoculated 24 hr after pruning, and 10 vines were inoculated 14 days after pruning. The vines were pruned 14 December 1990.

Experiment 91WA was a 3 × 2 factorial with a noninoculated control in a completely randomized design. The first factor was fungicide treatment. Treatments were benomyl, myclobutanil, and a nontreated control. A surfactant (Triton B-1956) was added to all fungicide suspensions (2 ml/L). The second factor was application method. For each wound treatment, 20 vines received the fungicide applied by paint brush, and 20 vines received the fungicide applied by a pneumatic sprayer, the Felco-matic Wasp pneumatic pruning shear (Felco S.A., Les Genevoys sur Coffrane, Switzerland). This device sprays the chemical onto the wound surface in a fine mist as the pruning cut is made. Vines were pruned and treated 9 January 1991 and inoculated 11 January 1991.

Experiment 91WB was identical to experiment 90F, except that inoculations were performed 2 days and 14 days after pruning. Vines were pruned 30 January 1991.

Symptoms of Eutypa dieback do not appear for 2–4 yr after infection (15). Therefore, an alternative method for assessing infection was necessary. The method of Petzoldt et al (22,23) was followed, with a few modifications. Spurs were collected during the next dormant season after inoculation. They were excised from the vines and brought to the laboratory for reisolation of the pathogen. The bark was stripped from each spur, and the spur was split longitudinally. Each half was cut into sections about 1 cm in length and placed in 0.5% NaOCl for 3 min for surface disinfestation. The pieces were removed, blotted dry on a clean paper towel, and placed in petri dishes on PDA amended with 100 μg/ml of streptomycin sulfate, 50 μg/ml of chlortetracycline HCl, and 5 μg/ml of dicloran. The dishes were incubated for 5 days at room temperature, at which time they were inspected for mycelial growth. *E. lata* mycelium can be identified by its color, colony morphology, and growth rate when compared with a known culture of the same age (22,23). If *E. lata* was detected growing from any section of a

Table 1. EC_{50} values (μg/ml) for inhibition of *Eutypa lata* radial growth by fungicides[a]

Fungicide	Isolate		
	GC13[a]	GC16[c]	GA17[d]
Vinclozolin	6.27 a	3.94 c	3.75 cd
Iprodione	4.99 b	3.82 cd	2.42 d
Triadimefon	4.01 c	5.01 b	1.49 e
Fenarimol	0.54 g	0.77 f	0.42 hi
Myclobutanil	0.35 i	0.46 gh	0.40 hi
Benomyl	0.09 j	0.11 j	0.09 j
Flusilazole	0.05 j	0.03 j	0.05 j

[a] Values presented are means of three experiments. Values followed by the same letter are not significantly different, as determined by Duncan's multiple range test ($\alpha = 0.05$).
[b] Isolated from a cankered Cabernet franc vine in Sonoma County, California.
[c] Isolated from a cankered French Colombard vine in Merced County, California.
[d] Grown from ascospores from a Sauvignon blanc vine in Napa County, California.

given spur, that spur was counted as infected. The proportion of the five spurs that was infected was recorded for each vine. The mean proportion of infected spurs was then calculated for each treatment. Two-way analysis of variance was performed on the arcsine square-root transformed proportions, and mean separation was executed by DMRT. Standard deviations for each treatment and plots of residual versus predicted values were examined to confirm uniformity of variance. Noninoculated controls were not included in the analyses.

Precipitation was monitored for 15 days after pruning in each experiment.

RESULTS

In vitro experiments. The growth rates of the three isolates were similar on PDA, although GA17 consistently grew slightly faster than the others. The EC_{50} values for inhibition of mycelial growth (Table 1) were significantly affected by both fungicide and isolate ($P = 0.0001$), and there was a significant interaction between the two effects ($P = 0.0001$). Benomyl and flusilazole had significantly ($\alpha = 0.05$) lower EC_{50} values (0.1 μg/ml or less) than the other fungicides. Fenarimol and myclobutanil had EC_{50} values between 0.35 and 0.77 μg/ml, significantly lower ($\alpha = 0.05$) than iprodione, triadimefon, and vinclozolin, which had relatively high EC_{50} values, ranging from 1.49 to 6.27 μg/ml. The mean EC_{50} value was highest for isolate GC13 and lowest for isolate GA17.

Ascospore germination on PDA after 48 hr was consistently higher than 90%. The EC_{50} values for inhibition of germination for fenarimol, flusilazole, myclobutanil, and triadimefon were 1.10, 0.06, 1.02, and 11.42 μg/ml, respectively. Flusilazole was significantly more effective than the other fungicides, and fenarimol and myclobutanil were significantly more effective than triadimefon, as determined by DMRT ($\alpha = 0.05$). At the highest fungicide concentration used in this study (10 μg/ml), benomyl, iprodione, and vinclozolin achieved only 26, 8, and 7% inhibition of germination, respectively. Since iprodione and vinclozolin were obviously less effective against germination and growth than the other fungicides, they were not included in the field experiments.

No *E. lata* isolates were detected that were capable of growth on 10 μg/ml of benomyl. A high proportion of ascospores germinated on this medium, but none grew into colonies.

Field experiments. In experiment 90S, fungicide treatment had a significant effect ($P = 0.0012$) on the percentage of wounds infected. The effect of vineyard block and the interaction between block and treatment were not significant ($P = 0.9496$ and 0.8597, respectively). As determined by DMRT ($\alpha = 0.05$), all four fungicides reduced infection compared with the untreated control (Fig. 1). The lowest proportion of infected wounds occurred in the benomyl treatment, but fenarimol and myclobutanil were not significantly different from benomyl (DMRT, $\alpha = 0.05$). Benomyl reduced infection from 43 to 19%, for a 56% reduction. The mean percent infection for the noninoculated treatment was 2%.

In experiment 90F, fungicide treatment and inoculation date both had a significant effect on infection ($P = 0.0001$ and 0.0480, respectively). Infection was higher for the second inoculation date in each of the fungicide treatments except flusilazole. Fenarimol and triadimefon failed to reduce the amount of infection significantly compared with the untreated control for both inoculation dates (DMRT $\alpha = 0.05$). Myclobutanil moderately reduced infection, whereas benomyl and flusilazole were very effective and not significantly different from each other on both inoculation dates (Fig. 2A). In the best treatments, benomyl and flusilazole, the percent reduction in infection was close to 80% for both inoculation dates (Fig. 2B). The mean percent infection in the noninoculated treatment was 24%.

In experiment 91WA, fungicide treatment was significant ($P = 0.0001$), and there was no significant difference between the two application methods ($P = 0.9225$). The interaction also was not significant ($P = 0.1950$). Thus, the pneumatic sprayer–pruner was as effective as hand application for both fungicides. Mean separation indicated there was no significant difference between benomyl and myclobutanil (DMRT, $\alpha = 0.05$), which were both very effective (Table 2). No infection was detected in the noninoculated treatment.

In experiment 91WB, fungicide treatment was significant ($P = 0.0001$), and inoculation date was not ($P = 0.7607$). However, there was a highly significant interaction between fungicide treatment and inoculation date ($P = 0.0001$). Infection in the untreated controls that were inoculated after 2 wk was significantly less than in those that were inoculated after 2 days (Fig. 3A). All five fungicides reduced infection significantly on both inoculation dates. There were no significant differences among the fungicides except that, for the second inoculation date, the percentage of infected wounds with flusilazole treatment was significantly lower than with triadimefon and fenarimol. There were no differences in percent wounds infected between inoculation dates for the fungicide treatments. However, when the data were transformed to percent reduction in infection, some differences appeared. Benomyl, fenarimol, and triadimefon were significantly less effective for the second inoculation date than for the first inoculation date (Fig. 3B). The reduction in infection was significantly less (DMRT $\alpha = 0.05$) for the second inoculation date than for the first inoculation date for all fungicides except flusilazole. The mean percent infection for the noninoculated treatment was 6%.

In experiment 90S, it rained several millimeters during the night following the inoculations (Fig. 4A). In experiment 90F, nearly 10 mm of rain fell during the night following pruning, before the inoculations (Fig. 4B). In experiment 91WA, there was no significant precip-

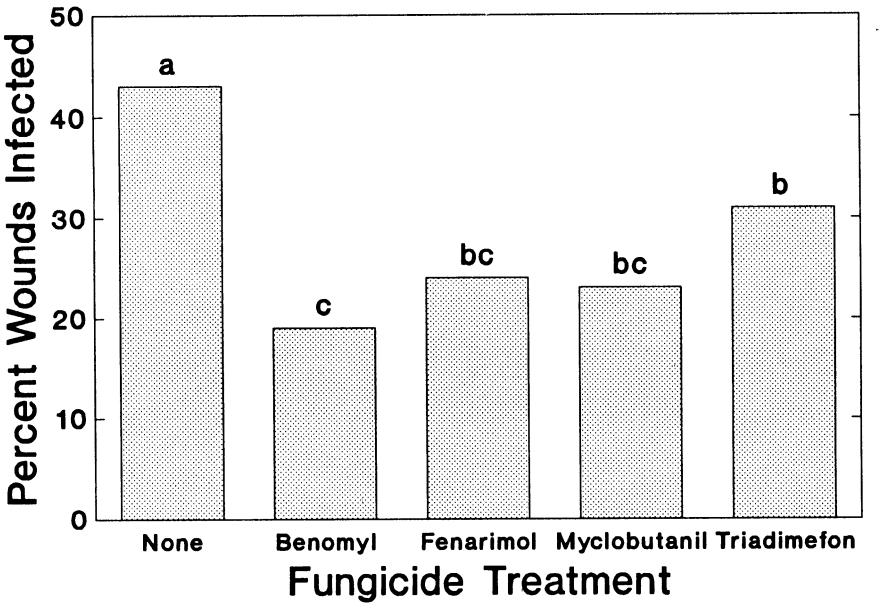

Fig. 1. Mean percentage of wounds infected for experiment 90S, performed during the spring of 1990. Values are means for 10 vines per treatment per block. None = inoculated, nontreated control. Bars with the same letter were not significantly different ($\alpha = 0.05$), according to Duncan's multiple range test. Data are combined for the two experimental blocks. Vines were pruned and treated with fungicides on 8 March 1990, and each wound was inoculated with 10^3 *Eutypa lata* ascospores on 9 March 1990.

itation for 15 days after pruning (Fig. 4C). In experiment 91WB, there was heavy precipitation for several days after the inoculations (Fig. 4D).

DISCUSSION

In this study, flusilazole was highly effective in both field experiments in which it was included. Benomyl was highly effective in three experiments and only moderately effective in one experiment. Myclobutanil was highly effective in two experiments and moderately effective in two experiments. Fenarimol and triadimefon were highly effective in one experiment, moderately effective in one, and ineffective in one. The relatively poor performance of fenarimol and triadimefon, especially when subjected to precipitation, indicate that these chemicals probably are not practical alternatives to benomyl for grapevine pruning wound protection.

In general, EC_{50} values for radial growth were good predictors of the relative efficacy of the fungicides in the field. However, the differences in EC_{50} values were small compared to the differences in efficacy in the field. The poor efficacy in the field for some of the fungicides may have been due to some characteristics of the formulations used, rather than a lack of toxicity to the fungus. Ability of the fungicides to inhibit ascospore germination was not consistently correlated with field efficacy. For example, benomyl inhibited germination poorly but was among the most effective fungicides for reduction of infection in the field.

The pneumatic pruning shear was an effective alternative to hand application of the fungicides. Similar results were reported by other researchers investigating Eutypa dieback of apricot (3,6,7). Large commercial operations that use pneumatic pruning might save labor costs by combining the pruning and wound treatment operations. The unit can cut grapevine canes up to 3 cm in diameter, which would include most cuts made during routine pruning. Larger cuts made with a saw during retraining or sanitation would still need to be painted with the fungicide. The unit has a high initial cost, but this might be offset by the savings in labor costs.

Precipitation seemed to influence the efficacy of some of the fungicides in the field experiments. This was most obvious in experiment 90F. Approximately 9.4 mm of precipitation fell during the night following the fungicide application,

Table 2. Mean percentage of wounds infected by *Eutypa lata* after fungicide treatment in experiment 91WA[a]

	Application method[b]	
Fungicide	**Brush**	**Spray**
Benomyl	19	15
Myclobutanil	9	14
Control	82	76
Mean	33	35

[a] Vines were pruned and treated with fungicides either by hand with a paint brush or by spray with the pneumatic sprayer–pruning shear on 9 January 1991. Each wound was inoculated with 10^3 ascospores of *E. lata* on 11 January 1991. Values presented are the means of 20 vines per treatment.

[b] There was no significant difference between application methods ($P = 0.9225$); the interaction also was not significant ($P = 0.1950$).

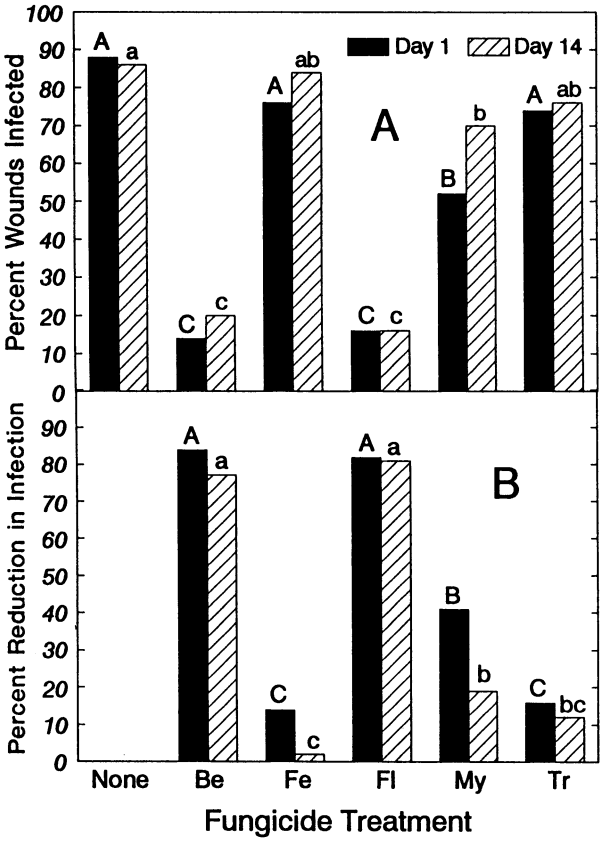

Fig. 2. (A) Mean percentage of wounds infected for experiment 90F, performed during the fall of 1990. **(B)** Mean reduction in percentage of wounds infected for each treatment in experiment 90F. Be = benomyl, Fe = fenarimol, Fl = flusilazole, My = myclobutanil, Tr = triadimefon, None = inoculated, nontreated control. Values are means for 10 vines per treatment. Vines were pruned and treated with fungicides on 14 December 1990, and each wound was inoculated with 10^3 *Eutypa lata* ascospores on either 15 December (solid bars) or 28 December 1990 (crosshatched bars). Within each inoculation date, bars with the same letter are not significantly different, according to Duncan's multiple range test ($\alpha = 0.05$).

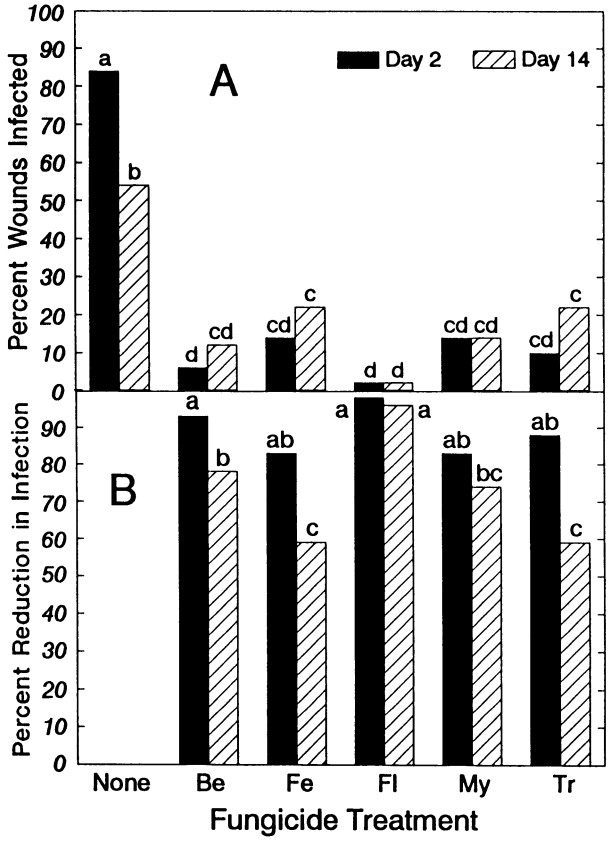

Fig. 3. (A) Mean percentage of wounds infected for experiment 91WB, performed during the winter of 1991. **(B)** Mean reduction in percentage of wounds infected for each treatment in experiment 91WB. Be = benomyl, Fe = fenarimol, Fl = flusilazole, My = myclobutanil, Tr = triadimefon, None = inoculated, nontreated control. Values are means for 10 vines per treatment. Bars with the same letter were not significantly different ($\alpha = 0.05$), according to Duncan's multiple range test. Vines were pruned and treated with fungicides on 30 January 1991, and each wound was inoculated with 10^3 *Eutypa lata* ascospores on either 1 February (solid bars) or 13 February 1991 (crosshatched bars).

before inoculation. In this experiment, fenarimol, myclobutanil, and triadimefon were much less effective than in the other field experiments. In experiments 90S and 91WB, precipitation occurred during the night following the first inoculation, but all fungicides provided good protection. When rainfall occurred before inoculation (experiment 90F and the second inoculation in experiment 91WB), it appeared to have a detrimental effect on the performance of most of the fungicide treatments. It is likely that the rainfall washed away some of the fungicide from the pruning wounds before the spores were applied, although we have no direct evidence for this. Since grapevines often must be pruned when rain is likely, an effective fungicide should remain active even after a heavy rain. Flusilazole was not influenced by precipitation.

Some of the conditions under which the experiments were conducted may have influenced the overall infection levels but should not have biased the results. For example, the inoculum level used in this experiment (10^3 spores/wound) was artificially high. This level of inoculum was used to ensure high levels of infection in the untreated vines and to facilitate detecting differences among the fungicides. Under natural conditions, it is unlikely that a wound would receive more than 10–100 spores (4,25). Also, inoculations were performed only 1 or 2 days or 2 wk after fungicide treatment, and conditions were not always favorable for infection. Under natural conditions, inoculum may arrive at any time, and wounds may remain susceptible for over 4 wk (19,23). In these experiments, in fact, wounds were subjected to natural inoculum in addition to the artificial inoculations. In experiments 90S and 91A, infection due to natural inoculum (noninoculated vines) was negligible. This may be attributed to the late pruning date in experiment 90S (23), and the lack of precipitation in the weeks following pruning in experiment 91WA (1). In the other two experiments, infection due to natural inoculum was higher, but under the conditions encountered in the experiments, benomyl and flusilazole effectively controlled natural infections as well as those due to inoculation. Therefore, it seems that the fungicides would perform better under natural conditions than under the high inoculum level used in these experiments.

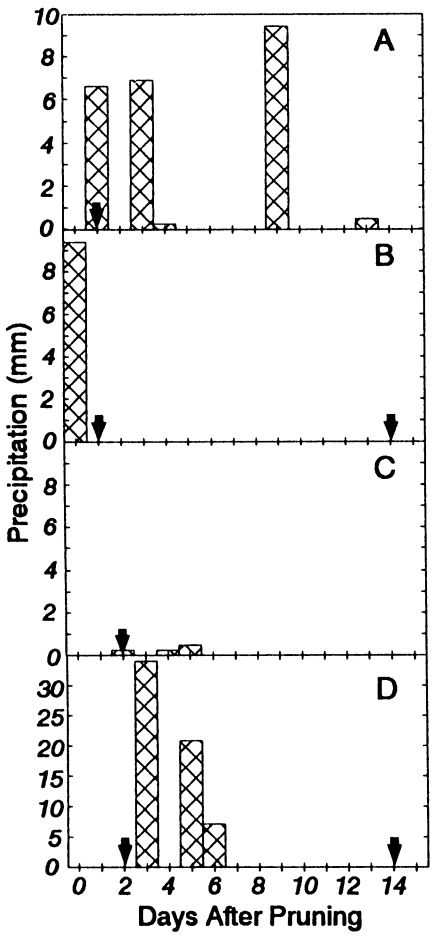

Fig. 4. Precipitation occurring for the first 15 days after pruning for **(A)** experiment 90S, **(B)** experiment 90F, **(C)** experiment 91WA, and **(D)** experiment 91WB. Arrows indicate days on which inoculations were performed.

We have not adequately explained the apparent ineffectiveness of benomyl that has been reported by grape growers (*unpublished*). Resistance to benomyl was not detected in this study, and its development seems unlikely, due to the long incubation period for *E. lata*, and the fact that most infections do not result in reproduction. In this study, fungicides were applied liberally to each pruning wound. It is possible that vineyard laborers do not carefully apply a sufficient coating of fungicide to every wound when a large number of wounds must be treated. Another possible reason for the apparent ineffectiveness of benomyl could be a lack of persistence on the wounds. Other researchers have reported a loss of efficacy over time when benomyl was applied to apricot pruning wounds (18,24). A similar result occurred in experiment 91WB. Benomyl was significantly less effective when wounds were inoculated 2 wk after fungicide application. The same was true in this experiment for fenarimol and triadimefon, and for myclobutanil in experiment 90F. This decrease in efficacy, coupled with long durations of wound susceptibility, may result in high levels of infection under some conditions. Only flusilazole did not decrease in efficacy in either experiment. Flusilazole, and possibly myclobutanil, could be effective alternatives to benomyl for grapevine pruning wound protection. The low EC_{50} values of flusilazole for both radial growth and germination indicate that this chemical may be as effective at lower concentrations, thereby providing an opportunity to reduce fungicide use and decrease the costs of managing Eutypa dieback.

ACKNOWLEDGMENTS

We thank the American Vineyard Foundation, the Andre Tselistcheff Scholarship Fund, and E. & J. Gallo, Inc., for funding this research.

LITERATURE CITED

1. Carter, M. V. 1957a. *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. 1957b. Vines aid spread of apricot "gummosis." J. Dep. Agric. South Aust. 59:178-184.
3. Carter, M. V. 1985. Evaluation of a manual spraying secateur for protecting trees and grapevines against wound-invading pathogens. Australas. Plant Pathol. 14:43-44.
4. Carter, M. V. 1991. The status of *Eutypa lata* as a pathogen. Int. Mycol. Inst. Phytopathol. Pap. 32.
5. Carter, M. V., Bolay, A., English, H., and Rumbos, I. 1985. Variation in the pathogenicity of *Eutypa lata* (=*Eutypa armeniacae*). Aust. J. Bot. 33:361-366.
6. Carter, M. V., and Mullett, L. F. 1978. Biological control of *Eutypa armeniacae*. 4. Design and performance of an applicator for metered delivery of protective aerosols during pruning. Aust. J. Exp. Agric. Anim. Husb. 18:287-293.
7. Carter, M. V., and Perrin, E. 1985. A pneumatic-powered spraying secateur for use in commercial orchards and vineyards. Aust. J. Exp. Agric. Anim. Husb. 25:939-942.
8. Carter, M. V., and Price, T. V. 1974. Biological control of *Eutypa armeniacae*. II Studies of the interaction between *E. armeniacae* and *Fusarium lateritium*, and their relative sensitivities to benzimidazole chemicals. Aust. J. Agric. Res. 25:109-115.
9. Carter, M. V., and Price, T. V. 1977. Explanation of the failure of a commercial scale application of benomyl to protect pruned apricot trees against Eutypa dieback disease. Aust. J. Exp. Agric. Anim. Husb. 17:171.
10. Duthie, J. A., Munkvold, G. P., Marois, J. J, Grant, S., and Chellemi, D. O. 1991. Relationship between age of vineyard and incidence of Eutypa dieback. (Abstr.) Phytopathology 81:1183.
11. English, H., and Davis, J. R. 1978. *Eutypa armeniacae* in apricot: Pathogenesis and induction of xylem soft rot. Hilgardia 46:193-204.
12. Gendloff, E. H., and Ramsdell, D. C. 1983. Fungicidal control of *Eutypa armeniacae* infecting Concord grapevine in Michigan. Plant Dis. 67:754-756.
13. Moller, W. J., and Carter, M. V. 1965. Production and dispersal of ascospores in *Eutypa armeniacae*. Aust. J. Biol. Sci. 18:67-80.
14. Moller, W. J., and Carter, M. V. 1969. A preliminary observation on apricot dieback prevention with chemicals. Plant Dis. Rep. 53:828-829.
15. Moller, W. J., and Kasimatis, A. N. 1978. Dieback of grapevines caused by *Eutypa armeniacae*. Plant Dis. Rep. 62:254-258.
16. Moller, W. J., and Kasimatis, A. N. 1980. Protection of grapevine pruning wounds from Eutypa dieback. Plant Dis. 64: 278-280.
17. 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.
18. Moller, W. J., Ramos, D. E., and Sanborn, R. R. 1977. Eutypa dieback in California apricot orchards: Chemical control studies. Plant Dis. Rep. 61:600-604.
19. Munkvold, G. P. 1992. Epidemiology and control of Eutypa dieback of grapevines. Ph.D. dissertation. University of California, Davis.
20. Munkvold, G. P., and Marois, J. J. 1991. Fungicidal and biological control of Eutypa

dieback of grapevines. (Abstr.) Phytopathology 81:1346.
21. Pearson, R. C. 1982. Protection of grapevine pruning wounds from infection by *Eutypa armeniacae* in New York State. Am. J. Enol. Vitic. 33:51-53.
22. Pearson, R. C. 1986. Field evaluation of fungicides for control of diseases of grapes. Pages 168-174 in: Methods for Evaluating Pesticides for the Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
23. 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.
24. Price, T. V., and Carter, M. V. 1975. The persistence of benomyl in pruned apricot sapwood. Aust. J. Agric. Res. 26:529-536.
25. Ramos, D. E., Moller, W. J., and English, H. 1975. Production and dispersal of ascospores of *Eutypa armeniacae* in California. Phytopathology 65:1364-1371.
26. SAS Institute. 1985. SAS User's Guide: Statistics. Version 5 ed. SAS Institute, Cary, NC.