Efficacy of Natural Epiphytes and Colonizers of Grapevine Pruning Wounds for Biological Control of Eutypa Dieback

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ABSTRACT

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Bacteria and fungi that naturally colonize grapevine pruning wounds were evaluated for their ability to inhibit infection of grape wood by *Eutypa lata*. Of 770 isolates, 53 reduced infection of excised wood by more than 80%. Twelve of these isolates were tested under field conditions with artificial inoculations with the pathogen. Seven isolates significantly reduced infection in the field in at least one experiment. Consistent control was achieved with only two isolates, *Fusarium lateritium* and *Cladosporium herbarum*. These fungi were as effective as benomyl in reducing infection by *E. lata* under some conditions. These two isolates were more effective when inoculation with *E. lata* was delayed until 14 days after application of the wound colonizers, reducing infection by as much as 71%. Application of the wound colonizers with a pneumatic spraying secateur was as feffective at reducing as was application with a paint brush.

Additional keywords: canker, *Eutypa armeniacae*.

Eutypa dieback of grapevine, caused by *Eutypa lata* (Pers:Fr.) Tul. & C. Tul. (=*E. armeniacae* Hansf. & M.V. Carter), is a chronic, lethal disease affecting most vineyards in northern California that are over 15 yr of age (15). Eutypa dieback is difficult to control. The disease is spread by airborne ascospores, which are discharged during rain events and infect through pruning wounds (2). Inoculum can originate from a number of sources. Apricot and cherry orchards and other vineyards are the most likely inoculum sources (18,23), but *E. lata* also has been reported from a number of other cultivated and native woody species (4). Thus, inoculum often comes from outside the vineyard and is impossible to eliminate. Therefore, wound protection with a fungicide or wound sealer is recommended. Benomyl has been effective in experiments (12,16,20) and is the only fungicide registered in the United States for this purpose. Because each

wound must be treated individually, wound protection is a costly procedure. In California, many growers have reported little reduction in Eutypa dieback despite years of fungicide use. Pruning wounds can be susceptible for more than 4 wk (18), depending on the conditions, and benomyl may not be effective for this duration (10,17,18,22). Vines pruned later in the dormant season are less susceptible (21), but this practice is only partially effective, and many growers must prune throughout the dormant season due to labor considerations. Vineyard life can be prolonged by sanitation pruning, in which infected portions of the vine are removed and replaced by training new canes. This is performed sporadically in most affected vineyards. Sanitation pruning also is a costly procedure, and control methods that prevent infection would be preferable.

Biological control of pathogens that infect woody plants through wounds has been successful. The fungus *Peniophora gigantea* (Fries) Massee has been used as a biocontrol agent for *Heterobasidion annosum* (Fr.:Fr.) Bref. (24), which infects forest trees through cut stumps and spreads to adjacent trees via root grafts. Similarly, *Trichoderma harzianum* Rifai was used as a pruning wound dressing on orchard trees to protect against infection by *Chondrostereum purpureum* (Pers.:Fr.) Pouzar, the causal agent of silver leaf disease (13). Biological control of Eutypa dieback of apricot and grape also has been investigated. On apricot, *Fusarium lateritium* Nees. was applied to pruning wounds and reduced infection by *E. lata* (3,8,9). A mixture of *F. lateritium* and benomyl was found to be more effective than either component alone. Benomyl was believed to provide good short-term protection until *F. lateritium* had thoroughly colonized the wounds, providing good long-term protection (9). On grape, *Bacillus subtilis* reduced infection by *E. lata* when applied to fresh pruning wounds (11).

The objective of this research was to evaluate the ability of microorganisms that naturally occur on grapevine pruning wounds to prevent infection by *E. lata*. There is some evidence that natural colonization of pruning wounds by nonpathogenic microorganisms contributes to reduced infection of apricot and grapevine by *E. lata* (3,18). However, there is little information on the relative effectiveness of individual wound epiphytes or colonizers or on the potential for reducing wound susceptibility further by altering the population structure of the existing wound microflora. Preliminary results have been reported (19).

MATERIALS AND METHODS

Isolation of microorganisms. All isolates were collected from pruning wounds on 1-yr-old canes in a *Vitis vinifera* L. 'Chenin blanc' vineyard in Sacramento County, CA. The vineyard was pruned 2 February 1989, and pruned spurs were chosen at random for isolation of microorganisms from pruning wounds. Forty wounds each were collected 0, 1, 5, 10, and 20 days after pruning. Wounds were collected by excising the pruned spurs from the vines and placing them in paper bags. Bags were placed in a cooler and brought to the laboratory for isolation. For each sampling date, epiphytes were isolated from the wound surface of 20 spurs, and wound colonizers were isolated from the wood below the wound surface of the other 20.

Fungi and bacteria were isolated by two different procedures. Bacteria were isolated on Difco nutrient agar (NA) (Difco Laboratories, Detroit, MI) amended with 40 μg/ml of cycloheximide. At each sampling date, five wounds were appressed to the surface of agar for 5 s, and the culture dishes were incubated at 22 C for 24 h. Colonies from these dishes were streaked onto additional culture dishes, from which individual colonies were transferred to culture tubes. Five pruned spurs were stripped of their bark, placed individually in 50 ml of sterile phosphate buffer (1.0 mM KH_2PO_4, pH = 7.0), and shaken at 150 rpm on a rotary shaker for 1 h to isolate bacteria from the pruning wounds. A 100-μl aliquot was removed from each flask and spread onto NA. Dishes were incubated for 24 h at 22 C. Individual colonies from these plates then were transferred to culture tubes for storage. To isolate bacteria from within the wood, pruning wounds were surface disinfested for 30 s in 70% ethanol, and five holes (1 mm diameter, 5 mm deep) were drilled with a sterile drill bit into each of 10 pruning wounds. The wood shavings were collected in 25 ml of sterile phosphate buffer and placed in flasks on a shaker for 1 h. Aliquots of the buffer were spread on NA plates, and individual colonies were isolated, as above.

Fungi were isolated on potato-dextrose agar (PDA; Difco Laboratories) amended with 50 μg/ml of chlortetracycline HCl and 100 μg/ml of streptomycin sulfate. To isolate epiphytes, 10 wounds were pressed onto the agar surface and left in place for 24 h at 22 C. The wood was then removed from the agar, and the plates were incubated at 22 C until colonies appeared. Individual colonies were transferred to culture tubes and stored at 4 C. For isolation of fungi from within the wood, holes were drilled in the remaining 10 wounds, as described above, and the shavings were sprinkled directly onto PDA plates amended with antibiotics. Fungi growing from the wood shavings were transferred to culture tubes.

Laboratory screening. Of the 770 isolates, 391 bacteria and 348 fungi were screened in the laboratory for their ability to inhibit colonization of excised grapevine stems by *E. lata*. Isolates of *Botrytis cinerea* Pers.:Fr. and *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. were excluded because these fungi cause bunch rots of grapes. One-year-old canes were pruned from the same vineyard from which the isolates were collected. Canes were cut into segments approximately 1.5-cm long. Segments were autoclaved for 30 min to eliminate competition from other organisms, and the bark was removed. Segments were embedded upright in water agar in 9-cm-diameter culture dishes (10 per dish) and inoculated with 100 μl of a pruning wound epiphyte or colonizer suspension ($\geq 10^8$ propagules ml^{-1}). Twenty segments were inoculated with each of the organisms. Twenty segments were not inoculated and served as controls. Dishes were incubated for 48 h at 22 C. At this time, each segment was inoculated with 500 ascospores of *E. lata* in 50 μl of water. The segments were incubated again at 22 C. After 10 days, the segments were split longitudinally, and surface disinfested in 2.5% sodium hypochlorite for 12 min on a rotary shaker at 150 rpm. They were then placed on PDA and incubated for 5 days. The number of segments yielding *E. lata* was determined for each of the isolates, and the mean percent reduction in infection compared to the control was calculated for each isolate. Isolates that reduced infection of autoclaved wood by 80% or more were screened again using nonautoclaved wood.

The procedure described above was followed for the bacterial strains, but a slightly different procedure was followed for fungal isolates. Because many of the rapidly growing saprophytic fungi greatly inhibited infection of the autoclaved wood by *E. lata* in preliminary experiments, a more stringent screening method was required. This was identical to the procedure described above, except that the cane segments were not autoclaved.

Ten isolates were selected from those that most effectively inhibited infection of excised cane segments. The 10 isolates represented the range of taxonomic groups that were among the most effective isolates in the laboratory. These isolates were assigned to species based on morphology and/or fatty acid profiles (Microcheck, Inc., Northfield, VT).

Field evaluation. Two field experiments included 10 of these isolates, and one included only *F. lateritium*. The organisms were propagated on PDA (fungi) or NA (bacteria), and a cell suspension (10^8 propagules ml^{-1}) was prepared in phosphate buffer. The first field experiment was conducted in a 21-yr-old cane-pruned Thompson Seedless vineyard near Davis, CA. There were 13 treatments in a completely randomized design. Each treatment was replicated individually on 10 vines with five wounds per vine. Treatments were no inoculation, *E. lata* inoculation alone, benomyl at 1.25% a.i. (Benlate 50WP, E. I. Du Pont de Nemours & Co., Inc., Wilmington, DE), and 10 putative biocontrol isolates: 0b-001 (*Bacillus megaterium*), 0b-027 (*Micrococcus kristinae*), 1b-023 (*Pseudomonas fluorescens*, biotype B), 1bf-077 (*Rhodotorula rubra* (R. Demme) Lodder), 1bf-053 (*Candida famata* (Harrison) Meyer et Yarrow), 5bf-012 (*Aureobasidium pullulans* (de Bary) G. Arnaud), 5bf-039 (*Penicillium* sp.), 10bf-021 (*Cladosporium*

herbarum (Pers.:Fr.) Link), 10bf-069 (*A. pullulans*), and F1A (*F. lateritium*). The three bacterial strains originated as wound epiphytes, and the seven fungal isolates originated as wound colonizers. Vines were pruned on 5 February 1990, and the wound treatments were applied to runoff immediately with a paint brush. The next day, each wound was inoculated with 1,000 ascospores of *E. lata* in 50 μl of water. Wounds were on 1-yr-old canes.

The second field experiment was conducted in a Chenin blanc vineyard in Sacramento County, CA. The suspensions of wound colonizers and epiphytes were prepared in sterile water. This experiment included 17 treatments arranged in a completely randomized design. Ten putative biocontrol isolates were applied at pruning, and the wounds were inoculated with *E. lata* 2 days later. Two treatments were application of putative biocontrol isolates (F1A and 021) at pruning and inoculation with *E. lata* 14 days later. Two treatments were benomyl (1.25% a.i.) application at pruning and inoculation with *E. lata* either 2 or 14 days later. The remaining three treatments were controls, one noninoculated, one inoculated with *E. lata* after 2 days, and one inoculated with *E. lata* after 14 days. Two isolates from the previous year (*Penicillium* sp. and *B. megaterium*) were replaced with fungal isolates Tr1 (*Trichoderma viride* Pers.:Fr.) and 5f-020 (*Alternaria alternata* (Fr.:Fr.) Keissl.). These isolates were originally isolated as wound colonizers. Each treatment was replicated on 10 vines. Every wound on each vine received the wound treatments, but only five wounds per vine were inoculated with *E. lata*. The remaining wounds were sampled at weekly intervals for 3 wk to evaluate survival of the putative biocontrol isolates. Sampling was conducted only for fungal isolates. Spurs were excised, the bark was removed, and the spurs were surface-disinfested in 0.5% NaOCl for 2 min. The putative biocontrol agents were reisolated by placing wood chips from each spur (including the wound surface and the upper 1 cm of the spur) on PDA. The frequency of reisolation of biocontrol isolates was compared between treated and nontreated wounds. A chi-square test determined whether frequency of isolation was significantly greater in the treated wounds.

In the third field experiment, only *F. lateritium* was tested. This experiment included six treatments arranged in a completely randomized design. Each treatment was replicated on 20 vines with five wounds per vine. Treatments were no inoculation, inoculation with *E. lata* only, benomyl (1.25% a.i.) applied with either a paint brush or a pneumatic sprayer, and *F. lateritium* applied with either a paint brush or a pneumatic sprayer. The pneumatic sprayer was a Felco Felcomatic Wasp (Felco S.A., Les Genevoys sur Coffrane, Switzerland) spraying secateur (a pneumatic pruning secateur that sprays the wound treatment as the vines are pruned). All but the noninoculated treatment were inoculated with 1,000 ascospores of *E. lata* 2 days after pruning.

For each field experiment, the inoculations were left to develop in the field until the next dormant season. Because symptoms of Eutypa dieback do not develop for several years, infection must be evaluated by reisolation of the pathogen (21). Inoculated spurs were excised from the vines and brought to the laboratory for reisolation. The bark was stripped from the spurs, and the spurs were split longitudinally. They were then cut into chips approximately 0.5-cm long. The portion of the spur from the wound surface to the margin of the live and dead wood was included. The chips were surface disinfested in 0.5% sodium hypochlorite for 3 min and placed in culture dishes containing PDA amended with 50 μg/ml of chlortetracycline HCl, 100 μg/ml of streptomycin sulfate, and 5 μg/ml of dicloran. After 5 days of incubation, dishes were examined for growth of *E. lata*. If any chip from a spur yielded the pathogen, that spur was considered infected. Percent reduction in infection for each vine, P_v, was calculated by $P_v = 100(I_c - I_v)/I_c$, in which I_v is the proportion of infected spurs on vine v and I_c is the mean proportion of infected spurs for the appropriate control vines. Mean percent reduction in infection was calculated for each treatment. Analysis of variance and mean separation by Duncan's multiple range test (DMRT) were performed on the angular-transformed reductions in infection. Treatments from the third field experiment were compared using orthogonal contrasts on the angular-transformed proportions of infected spurs. Noninoculated controls were not included in data analyses.

RESULTS

A total of 379 fungal and 391 bacterial isolates were collected from grapevine pruning wounds. The most common fungi were classified in 12 genera or species: *Alternaria alternata* (35 isolates), *A. pullulans* (20 isolates), *Botrytis cinerea* (12 isolates), *C. herbarum* (31 isolates), *Diplodia* spp. (24 isolates), *Epicoccum nigrum* Link (21 isolates), *F. lateritium* (7 isolates), *Fusarium* spp. (38 isolates), *Penicillium* spp. (31 isolates), *Phomopsis viticola* (Sacc.) Sacc. (12 isolates), *R. stolonifer* (19 isolates), and *Ulocladium atrum* G. Preuss (22 isolates). Each of these fungi was isolated both as an epiphyte and as a wound colonizer, except *F. lateritium*, which was not isolated as an epiphyte. Sixty-two additional isolates of mycelial fungi (some unidentified) and 45 yeast isolates (unidentified) also were collected and screened. The relative numbers of fungal isolates in each genus did not necessarily represent the relative population sizes of organisms on the wounds. Bacterial strains were not generally characterized unless they were used in the field experiments, but *Pseudomonas* and *Bacillus* spp. were common. The sampling method was not quantitative, but qualitative differences were observed between common epiphytes and colonizers. Immediately after pruning, the diversity of both colonizers and epiphytes was low. For the next two sampling dates, the diversity of epiphytes was much greater than that of colonizers. *A. pullulans* and yeasts dominated the fungal flora among the colonizers. The diversity of colonizers versus epiphytes was similar for the remainder of the sampling dates. Bacteria were more numerous on the wound surface than in the wood

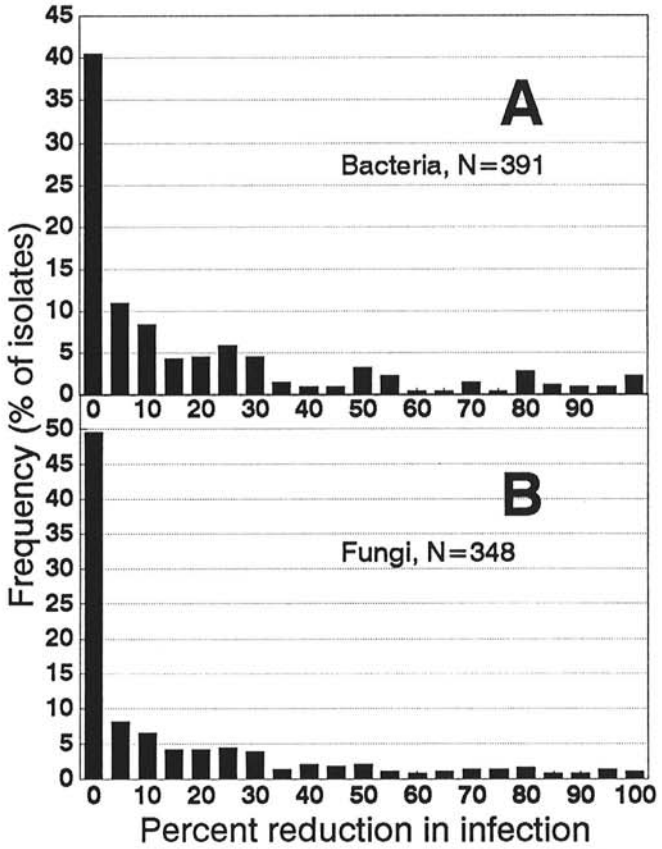

Fig. 1. Frequency distribution of **A,** bacterial strains and **B,** fungal isolates recovered from grapevine pruning wounds for reduction in infection of excised grape canes by *Eutypa lata*. There were 391 bacterial strains and 348 fungal isolates. Bacterial strains were applied to autoclaved cane segments, and fungal isolates were applied to nonautoclaved cane segments. One day after application of bacterial strains or fungal isolates, each segment was inoculated with 500 ascospores of *E. lata*.

tissue, except for the first sampling date, when very few bacteria were recovered.

Laboratory screening. Both fungal and bacterial isolates demonstrated a range of inhibition levels in the laboratory tests (Fig. 1). The wood segments inoculated only with *E. lata* were always infected. About 40% of the bacterial strains did not reduce infection of the autoclaved wood segments by *E. lata*. Another 19% reduced infection by 10% or less. Only 2% of the isolates completely inhibited infection by *E. lata* (Fig. 1A). When the 20 isolates with the best inhibition were screened again on nonautoclaved cane segments, they were slightly less effective in reducing *E. lata* infection (data not shown).

Of the fungal isolates, 49% did not reduce infection of the nonautoclaved wood segments by *E. lata*. Another 15% reduced infection by 10% or less, and only 1% of the isolates completely inhibited infection of grape wood (Fig. 1B). Twenty isolates that inhibited infection by 80% or more were screened a second time, and their reductions in *E. lata* infection were all within 10% of their original result (data not shown).

Field evaluation. In the first field experiment, infection levels were low; the inoculated, nontreated controls had a mean of 36% infection. Five fungal isolates significantly reduced infection compared to the *E. lata* inoculated control (Fig. 2). Two isolates, *F. lateritium* and *C. herbarum*, reduced infection to a greater extent (but not significantly different) than benomyl. Four additional fungal isolates were not significantly different from benomyl, according to DMRT. None of the three bacterial strains significantly reduced infection, and one (*B. megaterium*) resulted in a higher (but not significantly different) percent infection (40%) than the nontreated control. This strain was included in data analysis but is not shown in Figure 2. Infection in the noninoculated control was 2%.

In the second field experiment, infection levels were generally much higher than in the first experiment; mean percent infection in the inoculated, nontreated control was 84 and 54% when *E. lata* inoculations were conducted 2 and 14 days after pruning, respectively. Both wound treatment and time of inoculation had a significant effect ($P = 0.0001$) on percent reduction in infection. However, there also was a significant interaction ($P = 0.0017$) between treatment and time of inoculation. Benomyl had a significantly lower percent reduction in infection for the 14 day than for the 2 day inoculation. In contrast, the two biocontrol agents had a significantly higher percent reduction in infection for the 14 day than for the 2 day inoculation. Six fungal isolates reduced infection significantly ($\alpha = 0.05$) compared to the inoculated, nontreated control (Fig. 3). No isolates were as effective as benomyl for the 2 day inoculation. *F. lateritium* and *C. herbarum* were again the most effective of the biocontrol agents. These two isolates reduced infection as effectively as benomyl for the 14 day inoculation (DMRT, $\alpha = 0.05$). Two strains (*P. fluorescens* and *M. kristinae*) resulted in higher (but not significantly different) percent infection (86%) than the inoculated, nontreated control. These strains were included in the data analysis but are not shown in Figure 3. Infection in the noninoculated control was 6%.

Three weeks after wound treatment, *F. lateritium*, *C. herbarum*, *Alternaria alternata*, and *T. viride* were recovered from every treated wound that was sampled (Table 1). This high rate of reisolation was significantly different from the nontreated wounds, indicating that the application of these isolates resulted in successful colonization and survival on the wounds. The isolates that survived well also reduced infection by *E. lata* most effectively.

In the third field experiment, *F. lateritium* significantly reduced infection compared to the *E. lata* inoculated control, but benomyl reduced infection significantly more than *F. lateritium* (Table 2). There was no significant difference between application methods for either *F. lateritium* or benomyl. Infection in the noninoculated control was 0%.

DISCUSSION

Naturally occurring wound colonizers have a high potential for reducing infection of grapevines by *E. lata*. Several fungal isolates were as effective as benomyl, depending on the experiment and the inoculation time. Although benomyl is highly effective 2 days after fungicide application, its efficacy declines with time (10,17,18,22). In the second field experiment, efficacy of the biological control agents increased with time. Because grapevine pruning wounds can be susceptible to *E. lata* for 4 wk or more (18), the longer term protection afforded by biological control might be more beneficial than fungicidal protection, or the two

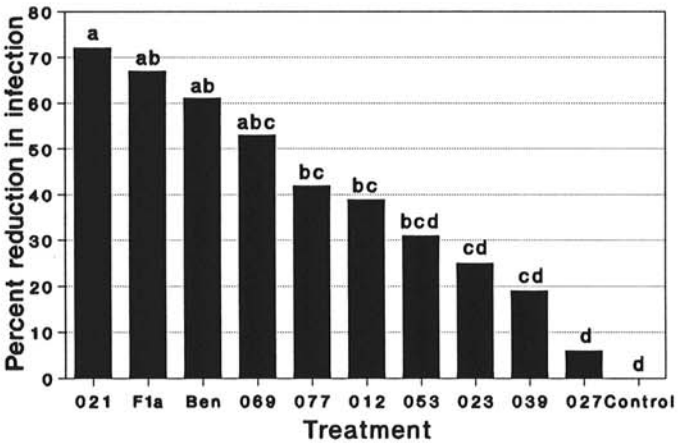

Fig. 2. Mean percent reduction in infection of wounds by *Eutypa lata* for biological control agents applied to grapevine pruning wounds in a Thompson Seedless vineyard near Davis, CA, in 1990. Biological control agents were applied immediately after pruning, and wounds were inoculated 24 h later with 1,000 ascospores of *E. lata* per wound. Treatments were 021 = *Cladosporium herbarum*; F1A = *Fusarium lateritium*; 069 = *Aureobasidium pullulans*; 077 = *Rhodotorula rubra*; 012 = *A. pullulans*; 053 = *Candida famata*; 023 = *Pseudomonas fluorescens*; 039 = *Penicillium* sp.; 027 = *Micrococcus kristinae*; Ben = benomyl; and Control = *E. lata* inoculation alone. *Bacillus megaterium* (not shown) resulted in more infection (but not significantly different) than the inoculated, nontreated control (Control), in which 36% of the inoculated wounds were infected. In the noninoculated control, 2% of the wounds were infected.

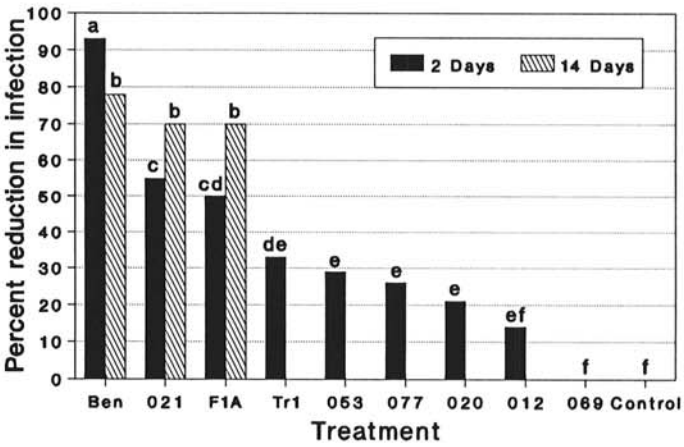

Fig. 3. Mean percent reduction in infection of wounds by *Eutypa lata* for biological control agents applied to grapevine pruning wounds in a cv. Chenin blanc vineyard near Courtland, CA, in 1991. Biological control agents were applied immediately after pruning, and wounds were inoculated either 2 or 14 days later with 1,000 ascospores of *E. lata* per wound. Treatments were 021 = *Cladosporium herbarum*; F1A = *Fusarium lateritium*; Tr1 = *Trichoderma viride*; 053 = *Candida famata*; 077 = *Rhodotorula rubra*; 020 = *Alternaria alternata*; 012 = *Aureobasidium pullulans*; 069 = *A. pullulans*; Ben = benomyl; and Control = *E. lata* inoculation after 2 days. *Pseudomonas fluorescens* and *Micrococcus kristinae* (not shown) resulted in more infection (but not significantly different) than the inoculated, nontreated control. In the control, 84 and 54% of the wounds inoculated after 2 and 14 days, respectively, were infected. In the noninoculated control, 6% of the wounds were infected.

methods could be employed in an integrated strategy. Also, the use of benomyl has been restricted or prohibited on many crops, so options must be developed for pruning wound protection if benomyl is unavailable in the future.

In previous research on Eutypa dieback in the United States, biological control has not been successful. *F. lateritium* did not significantly reduce infection of grapevines by *E. lata* in Michigan (12). This may be explained by the low inoculum dose used in the experiment (1,000 *F. lateritium* macroconidia per wound). Also, the isolate of *F. lateritium* was from Australia and may not have been adapted to the cold conditions in Michigan. *F. lateritium* was commonly isolated from grapevine pruning wounds in our study, and the isolate used in our experiments was from a California vineyard. *Trichoderma* spp. did not consistently suppress *E. lata* infection of apricot in California (14). In our experiments, *T. viride* was moderately effective at reducing infection of grapevine wounds by *E. lata* (Fig. 3).

The pneumatic spraying secateur was an effective application method for the biocontrol agents. Its use could reduce labor costs substantially for pruning wound protection. Previous research with Eutypa dieback of apricot produced similar results (6,7).

In the laboratory experiments, the majority of isolates reduced infection to some degree. Inhibition of *E. lata* was greater on autoclaved wood than on nonautoclaved wood. The isolates were not as effective in the field as in the laboratory. This may have been due to reduced colonization and survival. Temperature and humidity in the laboratory experiments were uniformly favorable for colonization by most of the isolates. In the field, temperature and humidity fluctuated substantially and were often lower than optimum. Low humidity may have retarded growth of the microorganisms and promoted rapid drying of the wounds.

In the laboratory screening, most of the highly effective isolates were wound colonizers. Although the same genera and species also were isolated from the wound surface, isolates from within the wood tissue may have been better adapted to colonization of the substrate. This characteristic would probably result in more effective exclusion of the pathogen. In the field, bacterial strains were not effective. Survival data were not collected for these strains, but they may not have survived well under harsh field conditions; also, they may be restricted to an epiphytic role in the field.

TABLE 1. Number of wounds on which putative biocontrol agents survived after application to grapevine pruning wounds in a cv. Chenin blanc vineyard in Sacramento County, CA[a]

	Days after application[b]		
Fungus	7	14	21
Fusarium lateritium			
Treated	10**	10**	10**
Nontreated	0	0	0
Cladosporium herbarum			
Treated	10*	9	10*
Nontreated	5	6	4
Trichoderma viride			
Treated	9**	9**	10**
Nontreated	0	0	0
Rhodotorula rubra			
Treated	8	8	6
Nontreated	5	4	3
Alternaria alternata			
Treated	10**	10*	10
Nontreated	1	5	6
Aureobasidium pullulans			
Treated	15	15	13
Nontreated	9	9	11

[a]No applications were made to nontreated wounds, which represent the level of natural colonization of pruning wounds by these organisms. Data represent survival on 10 treated and 10 nontreated wounds for each isolate, except *A. pullulans* (20 treated and nontreated wounds).
[b]* and ** indicate values that were significantly higher for the treated wounds than for the nontreated wounds according to the chi-square test ($\alpha = 0.05$ and 0.01, respectively).

In our experiments, the wounds were not covered after treatment, as other researchers have done (11). Covering the wounds prevents desiccation and promotes colonization by the biological control agents but does not mimic natural conditions. Some of our isolates survived quite well on the open wounds (Table 1). The isolates of *Alternaria alternata*, *C. herbarum*, *F. lateritium*, and *T. viride* survived on 100% of the wounds after 3 wk. The survival data in Table 1 are not quantitative and do not indicate the magnitude of the differences in colonization between the treated and nontreated wounds. These data indicate only the presence or absence of an organism on the wound. In many cases, the treated wounds were completely colonized by the organism that was applied, whereas the nontreated wounds may have had only a few small colonies. *C. herbarum*, in particular, can completely cover pruning wounds with sporulation after a large number of spores is applied. Conidia of *C. herbarum* are hydrophobic, and a dense layer of conidia and conidiophores may provide an effective physical barrier to pathogen spores carried in water droplets. In these experiments, the spores were applied in phosphate buffer or water. Suspending the spores in a nutrient solution that promotes sporulation on the wounds may increase the effectiveness of the isolates.

The mechanisms of antagonism are not known for either of these biocontrol agents. *F. lateritium* produces a diffusible compound in culture that inhibits growth of *E. lata*. This was reported previously (8) and was confirmed for our isolate in preliminary experiments (G. P. Munkvold and J. J. Marois, *unpublished data*). The compound has not been identified, and its production has not been demonstrated in vivo.

Rapid colonization of the wounds by the biocontrol agents may be the key to effective control through competition. Fresh wounds often are rich in nutrients but initially poor in microflora (1,18). They represent a virtually empty ecological niche. Fresh wounds lack the effective biological buffering that occurs in soil and inhibits the proliferation of introduced microorganisms. Microorganisms that arrive at the wound site early have the potential to preempt colonization by pathogens. This is the basis of biological control of *H. annosum* (24) and *Chondrostereum purpureum* (13). Domination of the substrate will occur only if the initial colonizer is well adapted to the substrate and is capable of rapid growth. Both *C. herbarum* and *F. lateritium* have these characteristics. In addition, both of these organisms have temperature optima of 21–22 C for growth, whereas *E. lata* has an optimum of 24 C. During the dormant season, temperatures in the field are commonly below 20 C, and the biocontrol agents are better adapted for growth at these temperatures. As they colonize the wounds, the pathogen probably is inhibited due to competition

TABLE 2. Mean percent infection and orthogonal contrasts for grapevine pruning wounds treated with benomyl (1.25% a.i.) or macroconidia of *Fusarium lateritium* (10^8 ml^{-1}) immediately after pruning, 11 January 1991[a]

Treatment	Percent infection
Control (*E. lata* inoculated)	79
Control (no inoculation)	0
F. lateritium	
Brush application	33
Spray application	39
Benomyl	
Brush application	19
Spray application	15

Orthogonal contrasts	Mean square	F	$P > F$
Control vs. treated	6.8289	156	0.0001
F. lateritium vs. benomyl	1.4281	32.7	0.0001
F. lateritium: brush vs. spray	0.0402	0.92	0.3401

[a]Each wound was inoculated with 1,000 ascospores of *Eutypa lata* after 48 h. Pruning wound treatments were applied by hand with a paint brush or by pneumatic spraying secateur (Felcomatic Wasp, Felco, Inc., Switzerland).

for space, moisture, and nutrition. Production of antibiotic compounds also may play a role in the cases of *F. lateritium* and *B. subtilis* (8,11).

The inoculum dose used in the field experiments (1,000 ascospores of *E. lata* per wound) is higher than can be expected under natural conditions (2,5,23). Fungicide efficacy should not be affected by pathogen population. However, the high inoculum dose may have reduced the efficacy of the biocontrol agents. In the interaction between pathogen and antagonist, their relative population sizes may strongly influence the success or failure of the antagonist. Therefore, it is possible that more effective biological control of Eutypa dieback would be observed under conditions of only natural inoculum.

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