Pathogenicity of Fungi Isolated from *Dendroctonus valens*, *D. brevicomis*, and *D. ponderosae* to Ponderosa Pine Seedlings

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ABSTRACT

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Two-year-old ponderosa pine seedlings were wound-inoculated with fungi isolated from the bark beetles *Dendroctonus valens*, *D. brevicomis*, and *D. ponderosae*. *Leptographium terebrantis* from *D. valens*, *Ceratocystis minor* from *D. brevicomis*, and *C. clavigera* from *D. ponderosae* each killed a high proportion of seedlings, whereas other fungal and control treatments did not. *L. terebrantis* caused greater mortality than either *C. minor* or *C. clavigera*. Seedlings killed by these fungi were bluestained, although the amount of stain varied. Surviving seedlings had large deposits of resin on the wound surface and in the xylem beneath the wound. Seedlings inoculated with sterile control blocks had less phloem necrosis, less xylem resinosis, and healed faster than did seedlings inoculated with fungi. Inoculating with *C. ips* in combination with *L. terebrantis* caused significantly less seedling mortality than did inoculating with *L. terebrantis* alone, indicating an inhibitory effect by *C. ips*. Similar effects were observed with combined inoculations of *C. ips* and *C. clavigera* or of *C. nigrocarpa* and *C. minor*. The imperfect form (Sporothrix) of *C. nigrocarpa* isolated from mycangia of *D. brevicomis* females had the same inhibitory effect on *C. minor*-caused mortality as did *C. nigrocarpa* isolated from either the surface of beetles or the phloem surrounding beetle galleries. Seedlings with smaller stem diameters had a higher mortality rate than did larger ones, and seedlings that had set bud apparently were more resistant to infection than seedlings that had just begun to elongate.

Additional key words: *Graphium* sp., mycangial basidiomycete, survival analysis.

Three species of *Dendroctonus* bark beetles frequently attack large ponderosa pines (*Pinus ponderosa* Laws.) concurrently: *D. brevicomis* LeConte (western pine beetle), *D. ponderosae* Hopkins (mountain pine beetle), and *D. valens* LeConte (red turpentine beetle). Of these three beetles, the western pine beetle is considered to be the primary cause of ponderosa pine mortality in the mixed conifer forests of the Sierra Nevada in California (12,26). Although a primary tree killer throughout its range, the mountain pine beetle typically is less aggressive than the western pine beetle in these forests. Under certain circumstances, the red turpentine beetle may be the first *Dendroctonus* sp. to attack trees, but it rarely kills them (13,26).

It has been demonstrated repeatedly that certain bark beetle-associated fungi can kill artificially inoculated hosts (5,7,15,19,22,23,27–29,43,44). Most of these studies have also shown that sizeable portions of the sapwood may be colonized and killed by these fungi even though the tree survives. Kaufmann and Stevens (20) found that ponderosa pines that survived attack by the mountain pine beetle were of lower vigor than unattacked trees, presumably because infection by bluestain fungi reduced functional sapwood.

It has been shown that a bark beetle may inoculate a tree with more than one fungus (e.g., 10), but the ecological roles of the different fungi are still largely a matter of speculation. The mountain pine beetle routinely transmits two bluestain fungi, *Ceratocystis ips* (Rumb.) C. Moreau and *C. clavigera* (Robins.-Jeff. & Davids.) Updhyay (35,41), whereas the western pine beetle transmits one bluestain, *C. minor* (Hedgc.) Hunt, and two nonstaining fungi, *C. nigrocarpa* Davids. and an unidentified basidiomycete (4,42). Three fungi are transmitted by the red turpentine beetle in California: *C. ips*, *Leptographium terebrantis* Barras & Perry (a bluestain), and a *Graphium* sp. (Owen, *unpublished*). Inoculation studies have demonstrated that *C. ips* and *C. minor* can kill trees and seedlings (5,7,22,23,28,29), and that *L. terebrantis* can kill seedlings (15,43). It is difficult, however, to draw conclusions on the relative pathogenicity of these fungi because of variability in the techniques used and the results obtained among studies.

Although more than one fungus may be transmitted by a given beetle, most inoculation studies have used single species as the inoculum. Studies using combinations of fungi were made by Bramble and Holst (7) and by Horntvedt et al (19). Neither study suggested that pairs of fungi might act synergistically in killing trees. Bramble and Holst (7) found some evidence that *Trichoderma lignorum* (Tode) Harz inhibited the ability of *C. minor* to kill shortleaf pine trees, but technique and sample size were insufficient to prove this. Horntvedt et al (19) killed Norway spruce trees by inoculating them with *C. polonica* (Siem.), *C. Moreau* or *C. polonica* with *C. penicillata* (Grosm.), *C. Moreau*, but not with *C. penicillata* alone. Whereas *C. polonica* penetrated into the sapwood of living trees when inoculated alone, *C. penicillata* was able to do this only when inoculated in combination with *C. polonica*.

The objectives of this study were: to test the relative pathogenicity to ponderosa pine seedlings of the fungi that are transmitted by the red turpentine, western pine, and mountain pine beetles and to test the pathogenicity of pairs of fungi from the same beetle species when coinoculated into seedlings.

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MATERIALS AND METHODS

During 1982–1984, seedlings were inoculated with fungi that are transmitted by *D. valens*, *D. brevicomis*, and *D. ponderosae*. Insects and galleries from which fungi were isolated all came from ponderosa pine in California.

One of the following media, with or without the addition of cycloheximide and streptomycin sulfate (17), was used in isolations: 1.75% potato-dextrose agar (PDA), water agar (WA), or 1% malt extract agar (MEA). Adult beetles were aseptically dissected into three parts (head, prothorax, and remainder of body) and each part was partially submerged in the medium. Pupae were plated in a similar manner but were not dissected. Pieces of phloem and wood were surface-sterilized 2–5 min in a 1:1:8 solution of 95% ethanol, commercial bleach, and water; rinsed with sterile water; and then aseptically cut into smaller pieces, which were plated on WA. Fungal mycelium and fruiting structures removed from pupal chambers, and eggs and frass from parent galleries were plated directly onto WA or MEA.

Fungi were stored in refrigerated culture less than 6 mo between the time they were isolated and when they were prepared for use in inoculations.

Twenty treatments were tested, including inoculation with a single fungus species, combinations of two fungus species from the same beetle, and a sterile control (Table 1). Pairs of fungi from different beetle species were not tested because different beetle species (and hence the fungi they carry) are often spatially isolated during colonization of the tree, especially during the early stages of beetle attack.

Each year, two new isolates per fungus species were used. For example, a total of six isolates of *L. terebrantis* were used: two in 1982, two in 1983, and two in 1984. Isolates were divided evenly among replicates of a particular treatment, and if a fungus was used in more than one treatment, the same isolates were used for each treatment. When a treatment consisted of two fungi inoculated in combination, all four possible combinations of treatments were equally replicated.

All isolates (see Table 1) were obtained from adult beetles or from phloem immediately surrounding beetle galleries in ponderosa pine and were derived from single hyphal tips. For 1982 tests, isolates came from *D. valens* (RTB) at Blodgett Forest or Boggs Mountain. Isolates were obtained from *D. valens*, *D. brevicomis* (WPB), and *D. ponderosae* (MPB) at Blodgett Forest and Yosemite Valley for 1983 tests and from Blodgett Forest for 1984 tests. Isolates labelled 'mycangial' (Table 1) were obtained from mycangia of female *D. brevicomis*. Spore masses were removed from mycangia with a sterilized minuten pin and streaked across WA to germinate. Because there is some question as to the identity of the mycangial hyphomycete (42), isolates of it were tested separately from isolates of *C. nigrocarpa* obtained from the body surface.

TABLE 1. Percent mortality of ponderosa pine seedlings when inoculated with various fungi vectored by *Dendroctonus valens* (RTB), *D. brevicomis* (WPB), and *D. ponderosae* (MPB)

Treatment[a]	Vector	Year of Test		
		1982	1983	1984
Lt	RTB	100.0 (40/40)[b]	98.2 (54/55)	70.0 (35/50)
Ci	RTB	2.5 (1/40)		
G	RTB	0 (0/37)		
Lt/Ci	RTB	85.0 (34/40)	96.4 (53/55)	8.0 (4/50)
Lt/G	RTB	97.1 (34/35)	90.9 (50/55)	
Ci/G	RTB	5.7 (2/35)		
Cm	WPB		71.1 (32/45)	40.0 (20/50)
Cn	WPB		2.2 (1/45)	
H	WPB		0 (0/45)	
B	WPB		2.2 (1/45)	
Cm/Cn	WPB		17.8 (8/45)	0 (0/50)
Cm/H	WPB		22.0 (10/45)	
Cm/B	WPB		75.5 (33/45)	
Cn/H	WPB		6.7 (3/45)	
Cn/B	WPB		0 (0/45)	
H/B	WPB		4.4 (2/45)	
Cc	MPB		75.0 (30/40)	56.0 (28/50)
Ci	MPB		7.5 (3/40)	
Cc/Ci	MPB		60.0 (24/40)	20.0 (10/50)
Control		7.5 (3/40)	2.5 (1/40)	0 (0/50)

[a] Lt = *Leptographium terebrantis*, Ci = *Ceratocystis ips*, G = *Graphium* sp., Cm = *C. minor*, Cn = *C. nigrocarpa* not from mycangium, H = mycangial hyphomycete, *C. nigrocarpa*, B = mycangial basidiomycete, Cc = *C. clavigera*.

[b] Proportion in parentheses.

For each year's test, 2-yr-old bare-root ponderosa pine seedlings were potted in a 1:1 mixture of peat moss and sand (U.C. mix) and grown for 2–4 mo in a greenhouse before inoculation. Seedlings were watered 1 or 2 times per week. Treatments were assigned to seedlings at random.

Inoculum blocks were prepared from ponderosa pine twigs approximately 1 cm in diameter (including bark) cut into 1- or 2-cm lengths. Blocks were boiled in a 10% malt extract broth for 2 hr (14), placed in glass jars with a small amount of WA at the bottom, and autoclaved for 1 hr at 121 C. Each jar (which contained 10–15 blocks) was inoculated with a single fungus-colonized agar plug and incubated 5 wk at room temperature (approximately 21 C). For paired-species treatments, the inoculum consisted of two 1-cm-long blocks (half blocks) each colonized by a different fungus. For treatments with a single fungus, two types of inocula were used. In 1982, a completely colonized 2-cm-long block (full block) was used, whereas in 1983 and 1984 the fungus-colonized block was 1 cm long and was combined with a 1-cm sterile block. A single 2-cm-long sterile block was used for control treatments.

Seedling stems were inoculated, using sterile technique, 4 cm above ground by cutting a 4- × 20-mm longitudinal wound to the surface of the sapwood and attaching inoculum blocks directly over the wound with Parafilm. For treatments requiring the application of two half blocks to the wound (i.e., combinations of two fungus species or one species with a sterile block), the positions of the two different blocks were alternated between the upper and lower halves of the wound.

Seedling mortality was recorded during the first 100 days after inoculation, which proved to be sufficient time to insure that all inoculum-related deaths had been observed. A seedling was considered dead when all its needles showed some discoloration (dull green, yellow, or brown). Tests were begun 19 May 1982, 19 May 1983, and 9 June 1984. In 1982 and 1983, seedlings were inoculated just after bud break, whereas in 1984 all seedlings had set buds by the time they were inoculated. As seedlings died, some were chosen for further study. Isolations were made from cross sections of the stem at, above, and below wounds by surface sterilizing a 1-cm section of the stem in a 1:1:8 solution of 95% ethanol, commercial bleach, and water for 1–2 min, washing with sterile water, and then aseptically removing a thinner section, which was plated on WA. Most isolations were made within 5 cm of wounds, but occasionally were made beyond this. At the end of a test, isolations were also made from a subset of the surviving seedlings.

Results of these experiments were analyzed using survival analysis (25), statistical methods for estimating, and comparing survival functions. A survival function expressed the distribution of survival times for seedlings exposed to the same treatment. In the 1982 and 1983 experiments, the Mantel–Cox test was used to compare survival functions of pairs of treatments. In 1984, seedling size, measured as stem diameter at height of inoculation, was included as a covariate in the analysis, using the Cox proportional hazards regression model. The Wald test was used to compare pairs of treatments and to test the significance of the regression on seedling size. BMDP Statistical Software (6) was used for computations.

Treatments from different years' experiments were not statistically compared because of concern about differences between seedlings, fungal isolates, and rearing conditions in

different years. Treatments that apparently had no effect, i.e., for which mortality was low, generally were not included in comparisons. Thus, the choice of treatments to compare was based on inspection of the data rather than on a priori considerations. Statistical tests were not adjusted to account for this because this study is viewed as exploratory, and we are willing to accept a relatively high overall error rate (cf. 24, chap. 1, p. 34).

RESULTS

Treatment effects. For each of the three beetle vectors, there was one fungus that, when inoculated by itself, caused high seedling mortality (Table 1). These three fungi were: *L. terebrantis* from *D. valens*, *C. minor* from *D. brevicomis*, and *C. clavigera* from *D. ponderosae*. All other single species treatments caused little or no mortality. In all years, *L. terebrantis* killed the largest proportion of inoculated seedlings. In the 1983 and 1984 experiments, *C. clavigera* and *C. minor* killed the second and third largest proportions of seedlings, respectively (Table 1). Survival of seedlings inoculated with *L. terebrantis* was statistically different from that of seedlings inoculated with *C. minor* in both 1983 and 1984, and from that of seedlings inoculated with *C. clavigera* in 1983 (Table 2). Survival of seedlings inoculated with *C. clavigera* was statistically different from that of seedlings inoculated with *C. minor* in 1984 (Table 2).

In seedlings that died after inoculation with *L. terebrantis*, *C. clavigera*, or *C. minor*, the pathogen was always isolated at the wound and at some distance from the wound, often at 5 cm or more away. Seedlings killed by these fungi consistently had some bluestain, although the amount of stain varied. Xylem directly beneath or adjacent to wounds often had limited resin-soaking and generally was not bluestained, suggesting that fungal colonization and/or penetration of such tissue was poor. This was in contrast to xylem opposite or away from wounds, which typically had no resin-soaking and might be extensively bluestained. Such a pattern indicates an active wound response by seedlings, but one that was insufficient to prevent fungal spread. Phloem near the wound was collapsed and completely brown, whereas farther from the wound, it could be turgid and healthy colored.

Seedlings that survived inoculation had large amounts of resin deposited on the wound surface and, depending on the treatment, in the xylem beneath the wound. Isolations typically yielded fungi only at the wound. In some seedlings, however, a longitudinally oriented strip of necrotic tissue extending beyond the wound also yielded fungi; in one seedling, *L. terebrantis* was isolated along the length of such a strip up to 15 cm above the wound. Control seedlings had the least amount of phloem necrosis and resinosis in the xylem and healed faster than seedlings inoculated with fungi (Fig. 1). Phloem necrosis and xylem resinosis tended to reflect particular fungal treatments. Inoculation with *Graphium* sp., for example, caused only slight tissue damage and response, while treatments with *L. terebrantis* caused considerably more damage and response.

In 1982 and 1984, inoculation of seedlings with *C. ips* and *L.*

TABLE 2. Statistical analyses of the effect of treatment on the survival of ponderosa pine seedlings: *p*-values for pairwise comparisons

Treatments[a] compared	Year of Test		
	1982	1983	1984
Lt vs Lt/Ci	*<0.0001*[b]	0.7808	*<0.0001*
Lt vs Lt/G	0.0783	0.2262	
Lt/Ci vs Lt/G	*0.0072*	0.3535	
Cm vs Cm/Cn		*<0.0001*	*<0.0001*
Cm vs Cm/H		*<0.0001*	
Cm vs Cm/B		0.4256	
Cc vs Cc/Ci		0.2630	*0.0002*
Lt vs Cm		*0.0076*	*0.0012*
Lt vs Cc		*0.0007*	0.1332
Cc vs Cm		0.1719	*0.0415*

[a] Lt = *Leptographium terebrantis*, Ci = *Ceratocystis ips*, G = *Graphium* sp., Cm = *C. minor*, Cn = *C. nigrocarpa* not from mycangium, H = mycangial hyphomycete, *C. nigrocarpa*, B = mycangial basidiomycete, Cc = *C. clavigera*.

[b] Null hypothesis: Survival curves for treatments are not different ($P<0.05$, reject null hypothesis). *P*-values less than 0.05 italicized. Mantel–Cox test used in 1982 and 1983. Wald test (permits inclusion of covariates into the analysis) used in 1984.

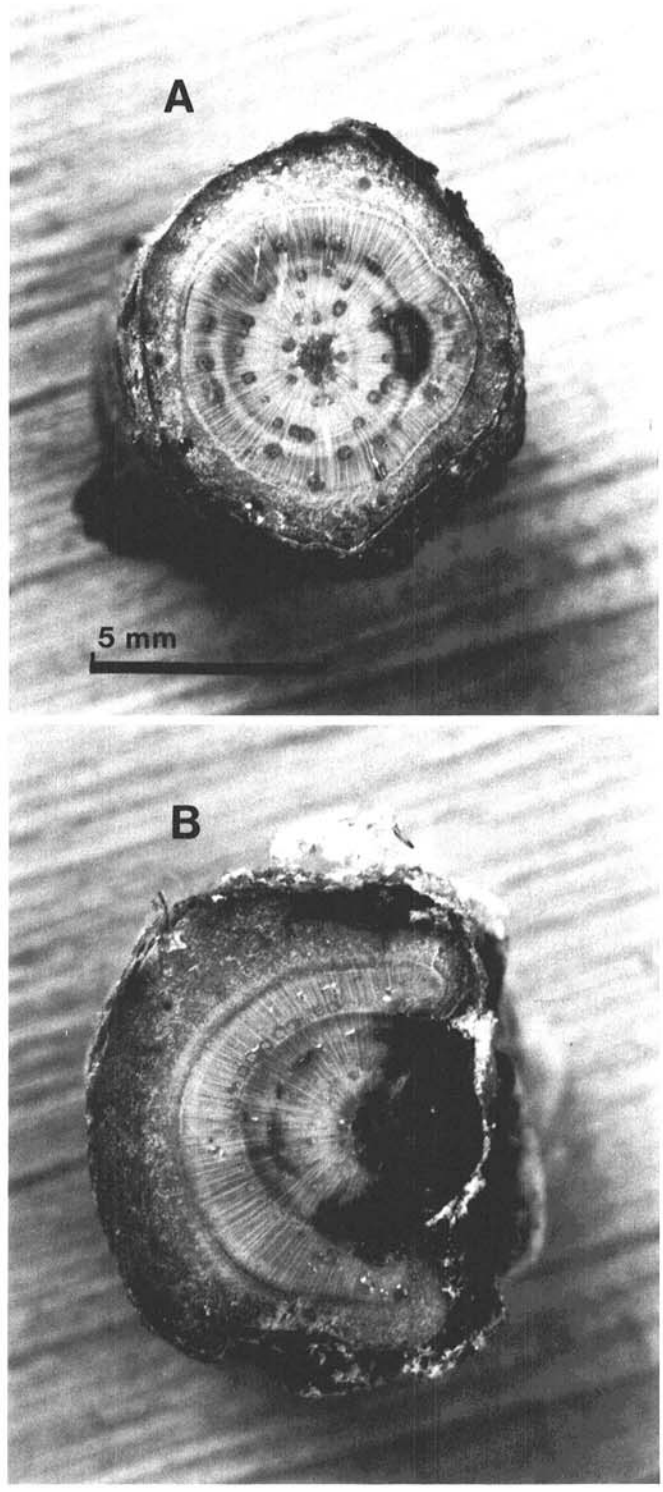

Fig. 1. Cross sections through the wounded portions of stems of ponderosa pine seedlings illustrating response to inoculation and degree of healing 11 mo after inoculation. Wounds are on the right side of the sections. Resin-soaked xylem appears darkened. **A,** Seedling inoculated with a sterile wooden block (control). **B,** Seedling inoculated with a block infected by *Leptographium terebrantis*.

terebrantis together resulted in a lower mortality than when seedlings were inoculated with *L. terebrantis* alone (Table 1). Similarly, in 1982 the combination of *C. ips* and *L. terebrantis* resulted in lower mortality than the combination of *L. terebrantis* and *Graphium* sp. These differences were statistically significant (Table 2). Clearly, *C. ips* inhibited *L. terebrantis*. Further, *C. ips* had a significant inhibitory effect on *C. clavigera* (1984 test), and *C. nigrocarpa* had a significant inhibitory effect on *C. minor* (1983 and 1984 tests). Hence, each of the three *Dendroctonus* spp. attacking ponderosa pine transmits one pathogenic fungus and another fungus that could inhibit the pathogen's ability to affect living host tissue. No evidence was found to indicate that fungi act synergistically in killing seedlings.

For treatments containing *L. terebrantis* in combination with either *C. ips* or *Graphium* sp., isolations from dead seedlings typically yielded *L. terebrantis* farther from the wound than either of the other two fungi. Isolations from 11 of 12 seedlings killed by the treatment *L. terebrantis*/*Graphium* sp. failed to yield *Graphium* sp. In the one seedling from which *Graphium* sp. was isolated, it was limited to the wound area. In contrast, *C. ips* was isolated from 11 of 14 seedlings killed by *L. terebrantis*/*C. ips*. *C. ips* was usually isolated away from the wound, but not ahead of *L. terebrantis* except in two seedlings. In these two, *C. ips* was limited to one side of the wound. Too few isolations were made from seedlings killed by *C. minor*/*C. nigrocarpa* and *C. clavigera*/*C. ips* to determine the distribution of fungi in them.

When fungi were cultured on artificial media (PDA, MEA, or WA) at room temperature, pathogenic fungi proved to be the fastest growing species. For fungi associated with *D. valens* the relative growth rates were *L. terebrantis* > *C. ips* > *Graphium* sp.; for fungi associated with *D. brevicomis* and *D. ponderosae* the relative growth rates were *C. minor* > *C. nigrocarpa* > mycangial basidiomycete, and *C. clavigera* > *C. ips*, respectively.

Mycangial hyphomycete. Isolations and inoculation tests in this study confirm that the hyphomycete commonly found in the mycangium of *D. brevicomis* females is an imperfect form of *C. nigrocarpa* (4,42). One of the difficulties in identifying *C. nigrocarpa* is that it does not readily produce perithecia in culture, and, if it does, the perithecia are usually poorly developed. Several isolates of the mycangial hyphomycete produced rudimentary perithecia in culture, although the majority did not. This same hyphomycete was isolated from the body surface of western pine beetles, and like the mycangial hyphomycete, the majority of these isolates were anascigerous. Those that produced mature perithecia were identified as *C. nigrocarpa*. Of 11 mycangial isolates obtained, one produced perithecia with ascospores and was also identified as *C. nigrocarpa*. In inoculation tests, both anascigerous and ascigerous isolates were used. In 1983, two of each type, and in 1984, one of each type were used. All isolates, whether from the body surface, mycangium, or the phloem, and whether ascigerous or anascigerous had a similar inhibitory effect on *C. minor* (Tables 1 and 2).

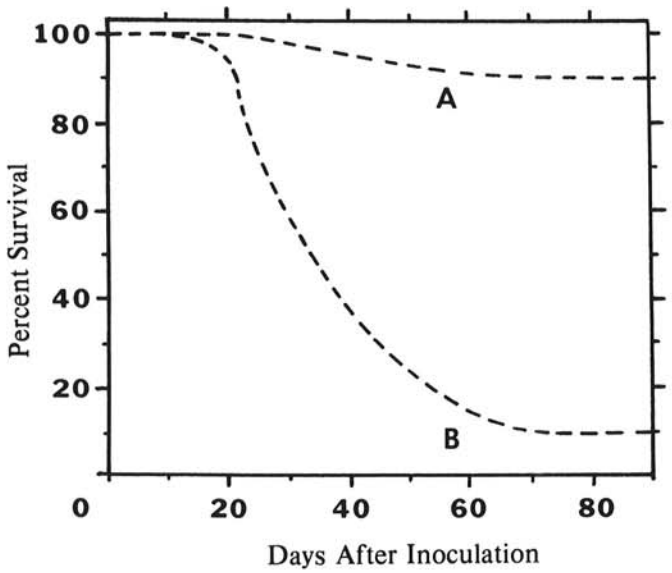

Fig. 2. Estimated survival curves for ponderosa pine seedlings with stem diameters of A) 10 mm and B) 4 mm inoculated with the same dose of *Ceratocystis clavigera*, 1984. Curves were plotted (6) using Cox's proportional hazards regression model.

Seedling diameter and phenology. When stem diameter at height of inoculation was included as a covariate in analyses of 1984 results, it proved to be significant (range of P values = 0.0008–0.0093, Wald test), explaining a large proportion of the variation in time to death. For all single and combined species treatments (except those that resulted in zero mortality), smaller diameter seedlings were more likely to die, and they died at a faster rate than larger diameter seedlings. This effect is illustrated in Figure 2. Because inoculum dose was constant regardless of seedling size, smaller seedlings received proportionally larger doses of inoculum, apparently enabling the pathogenic fungi to kill these seedlings more readily.

All 1984 treatments resulted in a dramatically lower percentage of mortality than the same treatments in 1982 and 1983 (Table 1). The 1983 test failed to demonstrate significant differences between *L. terebrantis* inoculated alone versus *L. tebrantis* inoculated with *C. ips*, and *C. clavigera* alone versus *C. clavigera* with *C. ips*, but in 1984 the differences were significant (Table 2). The inhibitory effect of *C. ips* on *C. clavigera* and *L. terebrantis* appears to have been enhanced by greater seedling resistance in 1984. The only notable difference between the test in 1984 and tests in other years was timing of inoculation in relation to seedling phenology. In 1984, seedlings had completed elongation and set buds by the time they were inoculated, whereas in 1982 and 1983 they had just broken bud and begun to elongate.

Another indication that seedlings were in a different physiological state when inoculated in 1984 was the manner in which some of them died. For 37 of the seedlings, the upper stem above the inoculation site died, while new growth occurred below the wound. For most of these seedlings, the new shoots below the wound eventually died also. Nine seedlings, however, survived via these basal shoots even though their tops were killed. This pattern of top killing coupled with a flush of sprouting below the wound was not observed in either 1982 or 1983.

DISCUSSION

Our finding that seedlings were more likely to die if inoculated during elongation than if inoculated after bud set supports a theory of tree resistance recently proposed by Lorio (21). He hypothesized that trees are more susceptible to southern pine beetle attack in the spring because photosynthates are being allocated to growth, and oleoresin production is low. Conversely, as photosynthate allocation shifts from growth to differentiation later in the season, oleoresin production and energy reserves increase, as does resistance to beetle attack. Also in agreement with this theory are the results of Paine (30). When ponderosa pines were inoculated with western pine beetle-associated fungi in May, he found that the size of the tree's response lesion was generally smaller than when inoculations were made in August or September. If the size of the lesion reflects the tree's capacity to resist infection, these results indicate a lower resistance to infection in the spring when height growth and earlywood production are occurring.

Given the tree killing abilities of *D. brevicomis* and *D. ponderosae*, it is notable that these beetles carry pathogens that are less virulent than the one carried by *D. valens*, a bark beetle that rarely kills trees. Assuming that these fungi play a role in helping the beetles overcome host defenses, the reverse might be expected.

Plausible explanations as to why *D. valens* transmits such a virulent pathogen relate to the location and density of the beetle's attacks. Vité (39) found ponderosa pine oleoresin exudation pressure to increase toward the base of the tree, where *D. valens* attacks. Our observations indicate that *D. valens* seldom attacks in high numbers or densities. Under these conditions, a less virulent pathogen may be unable to effectively colonize host tissues. By comparison, the western pine beetle initiates its attacks in the

upper midbole and attacks in much higher numbers and densities (26).

Because of the virulence of *L. terebrantis*, the introduction of this fungus into the tree by *D. valens* could result in damage to the tree's vascular system, even if the beetle fails to produce brood and the tree survives. The introduction of *L. terebrantis* into Scots pines (*P. sylvestris* L.) and Japanese black pines (*P. thunbergiana* Franco) by the black turpentine beetle apparently is a contributing factor to the mortality of these trees on Cape Cod, MA (18).

This study is the first demonstration of the ability of *C. clavigera* to kill an artificially inoculated host. Reid et al (34) inoculated lodgepole pine trees with both *C. clavigera* and *C. ips* and found that these fungi elicited a greater resinous reaction from the host than did sterile controls. *C. clavigera* was reported as the 'more aggressive' of the of the two fungi. Since then, many other studies have been conducted to examine the responses of lodgepole pine trees (31,33,36,37) or seedlings (38) to inoculation with *C. clavigera* but not *C. ips*. In none of these studies did inoculations cause host mortality.

Other studies (5,22,23) have demonstrated that *C. ips* can kill artificially inoculated hosts, but in our study, *C. ips*-caused mortality was low (1/40) and not much different from control mortality (3/40). Inoculation techniques used in previous studies were much more drastic than those used here; i.e., the stems were either completely or mostly girdled before the inoculum was applied. While *C. ips* may be pathogenic, our results show that it is not as virulent as *L. terebrantis*, *C. clavigera*, or *C. minor*.

The extent to which pathogenic bluestain fungi benefit bark beetles during their colonization of the tree is a matter of speculation. Hetrick (16) observed successful tree attack and brood development by *D. frontalis* Zimmermann in the apparent absence of bluestaining by *C. minor*. This phenomenon was frequent enough for him to conclude that *C. minor* is not essential to the beetle's success. Bridges et al (8) observed that this phenomenon was more common in areas of high southern pine beetle activity. Thus, they suggested that the pathogenic qualities of *C. minor* may be less important to the beetle's success when large beetle populations are available to attack trees. Raffa and Berryman (32) have shown through artificial inoculations of *C. clavigera* on lodgepole pine that an increase in the density of inoculations results in a decrease in monoterpene accumulation at individual inoculation sites. Christiansen (11) obtained a similar dose-dependent response by inoculating Norway spruce with *C. polonica*. These studies did not, however, include sterile control inoculations for comparison. Such a decrease in monoterpene accumulation would most likely have a positive influence on survival of attacking adults and the eggs that they lay in the tree. The conditions under which pathogenic bluestain fungi benefit bark beetles may be narrowly defined and probably are different for different beetle/fungus/host associations.

The degree to which pathogenic bluestain fungi might benefit attacking beetles by impairing the tree's defensive reactions could be offset by the fungi having a negative impact on brood development and survival. Barras (2), for example, found that *D. frontalis* broods were adversely affected when reared in bolts that were inoculated with *C. minor* before introducing parent beetles. Whitney (40) found that mountain pine beetle larvae feed in bluestain-free phloem during most of their development, indicating that bluestain fungi do not directly benefit the larvae. In contrast, Barras (3) presents evidence that nonstaining mycangial fungi from the southern pine beetle may aid brood development and survival.

Assuming that pathogenic bluestain fungi are detrimental to brood success, it would be important that mechanisms exist to limit the growth of these fungi once the death of the tree is secured. Our results indicate that certain other fungi carried by the beetles may, in part, serve this function. In ponderosa pine killed by the western pine beetle, bluestaining by *C. minor* is commonly observed as distinct and scattered patches on the surfaces of the sapwood (23), and in some cases may be absent from the tree (Vité and Rudinsky, in 23). Whitney and Cobb (42) reported that *C. minor* and the two nonstaining fungi, *C. nigrocarpa* and the mycangial basidiomycete, carried by *D. brevicomis* appear to be mutually exclusive in their colonization of the sapwood of beetle-killed trees. The inhibition of *C. minor* by *C. nigrocarpa* in seedling inoculations is probably a manifestation of the antagonism that apparently exists between these two fungi in nature.

By inoculating southern pine beetles with *C. minor* and then introducing them into pine bolts, Bridges and Perry (9) demonstrated that the development of bluestain in the bolts was greater if the beetles were surface sterilized before inoculation. Hence, one or more of the microorganisms (including mycangial fungi) that were removed from the beetles by surface sterilization apparently had a limiting effect on the growth of *C. minor*. Barras (1) noted that *Penicillium implicatum* Biourge was antagonistic to both *C. minor* and *C. ips*. In culture, a distinct zone of inhibition was produced between *P. implicatum* and these other fungi, indicating an antibiotic effect. While making isolations on artificial media, we noted no obvious zones of inhibition between various fungi listed in Table 1. This, however, does not exclude the possibility that antibiotic interactions were taking place or might take place under other conditions. Inhibitory effects could also be indirect.

The results of this and other studies support the hypothesis that pathogenic bluestain fungi serve a distinct, but limited role in the life history of bark beetles. *C. minor*, *L. terebrantis*, and *C. clavigera* each appear to be adapted for rapid, advanced colonization of host tissues during bark beetle attack on the tree. Apparently, these fungi are more tolerant of host-produced defensive compounds and/or better able to circumvent the effects of these compounds and thus colonize host tissue that other fungi cannot or are slow to colonize. The conditions under which these pathogens become established in the tree are not, however, static. As beetle colonization of the tree progresses, not only does host physiology change, but so does beetle behavior. Such changes may influence the sequence of establishment of nonpathogenic and pathogenic organisms.

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