# Variability in Virulence of *Heterobasidion annosum* Isolates from Ponderosa and Jeffrey Pine in Areas of High and Low Photochemical Air Pollution

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**ABSTRACT**

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Virulence of *Heterobasidion annosum* (= *Fomes annosus*) isolates from different geographic locations throughout California was evaluated by inoculation of ponderosa pine trees in the field and seedlings in the greenhouse. Tests were designed to compare isolates obtained from areas of chronic photochemical air pollution exposure with some from areas relatively free from pollution. Isolates displayed a wide range of virulence based on number of plants infected and rate of colonization. Percentage of infection varied from 4 to 74 in the greenhouse and from 0 to 50 in field tests. Colonization rates showed a 17-fold difference among isolates. However, relationships between geographic origin and level of virulence were not evident. Isolates from areas of relatively high, chronic air pollution exposure were generally as virulent as those from environments with little or no pollution.

Additional key words: *Pinus jeffreyi, P. ponderosa*, root disease

*Heterobasidion annosum* (Fr.) Bref. (= *Fomes annosus* (Fr.) Cke.) is an important root pathogen of conifers in north temperate forest ecosystems (2,10,12), but the effects differ from area to area because of various factors including hosts; tree vigor; and other stand, site, and environmental influences. Strains of the fungus exhibiting variable pathogenicity or virulence have been identified in inoculation experiments both in Europe (3) and the southeastern United States (7–9), but the differences were not related to environment. Differences in fungus virulence could be important in determining levels of influence exerted by environmental factors in disease processes. For example, perhaps less virulent strains cause significant disease losses only under mitigating environmental influences, such as host injury and disease predisposition by photochemical air pollution. If so, these strains would be expected to cause little damage where host injury is absent. Air pollution could also directly affect isolate virulence by genetic alterations.

Experiments were designed to determine whether isolates obtained from areas of chronic photochemical air pollution exposure were as virulent on ponderosa pine (*Pinus ponderosa* Laws.) as isolates from areas relatively free from air pollution exposure. Tests were made in a field environment of negligible photochemical air pollution and under controlled greenhouse conditions.

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## MATERIALS AND METHODS

**Field inoculations.** Field tests were made on the Georgetown Divide, El Dorado National Forest, CA, at sites ranging in elevation from 1,090 to 1,680 m. Trees were either in 10-yr-old plantations or were mature individuals averaging about 50 cm diameter at breast height. Two *H. annosum* isolates from California were tested. One (SV) was obtained from a colony arising from spores trapped on a wood disk in an area of chronic photochemical air pollution exposure (San Bernardino National Forest), and the other (BL) was isolated from an infected ponderosa pine in an area of negligible air pollution (El Dorado National Forest).

Inoculation procedures were similar to those previously described (5). Roots were analyzed for *H. annosum* infection and colonization 10 mo after inoculation. They were excavated to the root collar and cut from the tree with a handsaw with blade swabbed with 95% ethanol before each cut. Roots were taken to the laboratory, split longitudinally with a sterile knife, wrapped in moistened newspaper, and incubated for 7 days at about 24 C. They were examined under the dissecting microscope (×20–80) for extent of *H. annosum* colonization as determined by presence of fungal hyphae and sporulation on the split surface. Proximal, rather than distal, colonization was used for assessing tree root susceptibility because the pathogen had to invade unsevered root tissue when moving toward the tree. Proximal

colonization rates (millimeters per month) of roots inoculated by the two isolates were compared using one-way analysis of variance.

**Greenhouse inoculations.** Ten *H. annosum* isolates from California were tested in inoculations of 1-0 ponderosa pine seedlings. Isolates were obtained from either ponderosa or Jeffrey pine (*P. jeffreyi* Grev. & Balf.). Five isolates were from areas of chronic photochemical air pollution exposure (San Bernardino National Forest), and five were from areas of negligible air pollution (El Dorado National Forest, Stanislaus National Forest, and Yosemite National Park) (Table 1).

Inoculum blocks about $1.7 \times 0.5 \times 0.5$ cm (0.4 $cm^3$) were prepared from pine stem sections. These were autoclaved at 121 C for 60 min in glass jars, allowed to cool, inoculated with a conidial suspension of *H. annosum*, and incubated in the dark for 10 wk at about 24 C.

Inoculation procedures were similar to those previously described (5), except that seedlings were transplanted during the process. Seedling stems just above the root collars were washed with sterile water, and incisions were made into the xylem with a sterile knife. The inoculum block was then inserted, and the entire inoculation point was washed with sterile water and wrapped with masking tape. Controls consisted of seedlings inoculated with sterilized pinewood blocks. After inoculation, seedlings were transplanted into pots containing equal portions of U.C. soil mix (1) and sterilized sand. Seedlings were watered when needed to avoid moisture stress.

Fifty seedlings per isolate, plus 50 controls, were inoculated, placed on greenhouse benches, and examined periodically for foliar discoloration. Seedlings with advanced decline symptoms were examined for *H. annosum* infection and colonization. Declining seedlings were washed thoroughly and cut longitudinally through the inoculation point with a sterilized knife to expose xylem tissues. They were then placed in plastic bags with moistened paper towels, incubated at about 24 C for 7–10 days, and examined (×10–80) for extent of *H. annosum* colonization. Presence of resin and callus tissue production at the inoculation site were also noted. All nonsymptomatic seedlings were examined 3 mo after inoculation. The Fisher-Yates test of significance in $2 \times 2$ contingency tables was used to compare infection percentages by different isolates, and the Studentized range test for multiple comparisons was used to determine significance of differences in colonization rates (millimeters per day).

**Table 1.** Infection and colonization of inoculated ponderosa pine seedlings by *Heterobasidion annosum* (= *Fomes annosus*) isolates from areas of high and low air pollution levels

| Isolate | Host location[w] | Geographic location[x] | Relative oxidant level | Percentage of infection[y] | Colonization rate (mm/day)[z] | Percentage of mortality |
|---|---|---|---|---|---|---|
| HB11 | JP stump | SBNF | High | 74 a | 0.83 a | 58 |
| JL1 | JP stump | SBNF | High | 58 ab | 0.76 ab | 52 |
| Y2 | PP tree root collar | YNP | Low | 48 ab | 0.78 ab | 32 |
| Y7 | PP tree root collar | YNP | Low | 34 bc | 0.64 b | 26 |
| 01 | JP tree root collar | SBNF | High | 32 bc | 0.18 c | 4 |
| B1 | PP tree root collar | EDNF | Low | 30 bc | 0.09 c | 10 |
| A2 | JP tree root collar | SBNF | High | 22 cd | 0.28 c | 4 |
| SV1 | JP stump | SBNF | High | 22 cd | 0.13 c | 2 |
| S1 | PP stump | SNF | Low | 8 cde | 0.06 c | 2 |
| B2 | PP stump | EDNF | Low | 4 de | 0.05 c | 2 |
| Control | ··· | ··· | ··· | 0 e | 0.00 d | 0 |
| Mean of inoc. tree | ··· | ··· | ··· | 33.6 | 0.38 | 19.2 |

[w] JP = Jeffrey pine, PP = ponderosa pine.
[x] SBNF = San Bernardino National Forest, YNP = Yosemite National Park, EDNF = El Dorado National Forest, and SNF = Stanislaus National Forest.
[y] Values within the column followed by the same letter are not significantly different ($P = 0.05$) using the Fisher-Yates test of significance in $2 \times 2$ contingency tables.
[z] Means within the column followed by the same letter are not significantly different ($P = 0.05$) using the Studentized range test for multiple comparisons.

**Table 2.** Infection and colonization of inoculated ponderosa pine roots by *Heterobasidion annosum* (= *Fomes annosus*) on the El Dorado National Forest

| Isolate source | Site of inoculations[a] | No. of roots inoculated | Infection (%)[b] | Colonization rate (mm/mo)[b] |
|---|---|---|---|---|
| SV (San Bernardino) | Lake Walton | 16 | 50 | 1.2 |
| | Mutton Creek | 5 | 40 | 0.8 |
| | Walton Plantation | 18 | 39 | 1.4 |
| | Edson Plantation | 15 | 33 | 1.0 |
| | Subtotals | 54 | 41 | 1.2[c] |
| BL (El Dorado) | Lake Walton | 17 | 0 | 0 |
| | Mutton Creek | 10 | 0 | 0 |
| | Walton Plantation | 16 | 13 | 0.1 |
| | Edson Plantation | 16 | 13 | 0.3 |
| | Subtotals | 59 | 7 | 0.2[c] |
| Totals | ··· | 113 | 23 | 1.0 |

[a] Trees at Lake Walton and Mutton Creek averaged about 80 yr old, and those in the plantations were 11 yr old.
[b] Proximal infection and colonization from inoculation point.
[c] Means are significantly different ($P = 0.01$) using one-way analysis of variance.

## RESULTS AND DISCUSSION

Results of field inoculations (Table 2) indicated difference in virulence between the two *H. annosum* isolates tested. The isolate from the San Bernardino National Forest (SV) caused more infections and significantly greater ($P = 0.01$) proximal colonization than did the isolate from the El Dorado National Forest. Differences in host susceptibility resulting from tree age and site location were not apparent.

Inoculation of ponderosa pine seedlings demonstrated large variations in virulence among the 10 *H. annosum* isolates tested (Table 1). The isolates could be separated into two pathogenic groups based on percentage of seedling mortality and colonization rate. The four most virulent isolates were from the San Bernardino National Forest (HB11, JL1) and Yosemite National Park (Y2, Y7). However, two of the less virulent isolates (A2, SV1) were also from the San Bernardino National Forest. No infection was found on control seedlings during the experiment.

Our results confirmed the existence of *H. annosum* strains in nature that differ substantially in virulence. However, isolate differences relative to geographic origin were not found. The differences encountered also cannot be explained on the basis of the substrate from which the isolates were obtained; isolates from stumps and trees were about equally virulent.

Isolates from areas of chronic photochemical air pollution exposure were generally as virulent as those from relatively unpolluted environments. Apparently, pathogenic capabilities of *H. annosum* have not been adversely affected by long-term air pollution exposures. On the other hand, other studies (5,6) have shown that pollution injury increases host susceptibility. Thus, the overall effect of pollutant exposure should be an increase in disease impact.

Most uninfected seedlings produced callus tissue and resinosis as responses to inoculation. Inoculation sites of these seedlings were also often colonized by *Trichoderma* spp., which are common *H. annosum* competitors (8,11). On the other hand, infected seedlings that were readily colonized had little resin or callus production. Infected trees usually died 30–50 days after inoculation.

Aggressive isolates appeared to be more successful in attacking seedlings because they quickly grew from inoculum blocks into root collar tissues before host defense mechanisms were effective. Also, they may have been more successful in competing with *Trichoderma* spp. for host substrate. Tree death probably occurred after fungal colonization of the cambium at the root collar (2,4); no wood decay was evident.

Unfortunately, virulence of *H. annosum* isolates in the field could not be predicted from cultural characteristics such as colony morphology, asexual sporulation, and growth rate. Virulent isolates apparently can only be identified through pathogenicity tests or possibly by determination of decay capacity (6). The results of seedling inoculations with isolates SV and JL tended to confirm differences reported (6) in rates of decay and stump colonization.

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