**Secondary spread of Heterobasidion annosum in white fir root-disease centers**

Matteo Garbelotto, Garey Slaughter, Tina Popenuck, Fields W. Cobb, and Thomas D. Bruns

**Abstract:** Tree mortality caused by *Heterobasidion annosum* Fr. (Bref.) in white fir (*Abies concolor* (Gord. & Glend.) Lindl.) often appears in clusters; symptoms in the infected trees include sapwood and heartwood decay in tree boles and roots. Although the pathogen can spread from tree to tree through root contacts, it is often confined to the initially infected trees or stumps. We devised a field inoculation study to determine comparative virulence of fungal isolates, rates and modes of fungal colonization, preferential direction of fungal colonization, and effect of root size on fungal growth in white fir roots. Fifty trees were inoculated with eight *H. annosum* isolates, and sampled at 4 and 12 months. *Heterobasidion annosum* caused purple-brown staining and incipient wood decay within 4 months. Isolates from stumps were as virulent as isolates from trees. Fungal colonization was less in smaller than larger roots. Fungal colonization was greater in the proximal (towards the bole) than in the distal direction. Most fungal colonization in the distal direction occurred during the 4 months postinoculation, while colonization in the proximal direction occurred throughout the year.

**Résumé:** La mortalité causée par *Heterobasidion annosum* Fr. (Bref.) chez le sapin argenté (*Abies concolor* (Gord. & Glend.) Lindl.) se entrouvait souvent la formation de trouées. Chez les arbres infectés, les symptômes incluent la carie du bois d’aubier et du bois de cœur dans le tronc et les racines. Même si le champignon pathogène peut se propager d’un arbre à l’autre par les greffes de racines, il est souvent confiné aux arbres ou aux souches initialement infectés. Nous avons conçu une expérience d’inoculation champ afin de déterminer la virulence relative d’isolats du champignon, le taux et le mode de colonisation du champignon, la direction préférentielle de la colonisation et l’effet de la dimension des racines sur la croissance du champignon sur le sapin argenté. Cinquante arbres furent inoculés avec huit isolats de *H. annosum* et échantillonnés après 4 et 12 mois. *Heterobasidion annosum* a causé une coloration brun-pourpre et un début de carie en moins de 4 mois. Les isolats qui provenaient de souches étaient aussi virulents que ceux qui avaient été récoltés sur des arbres. La colonisation était plus faible dans les petites racines que dans les grosses. Elle était plus forte en direction du tronc que vers l’extérieur. La plus grande partie de la colonisation vers l’extérieur est survenue pendant les 4 mois qui ont suivi l’inoculation, tandis que la colonisation vers le tronc se poursuivait pendant toute l’année.

[Traduit par la Rédaction]

**Introduction**

Annosus root disease is caused by the basidiomycete *Heterobasidion annosum* Fr. (Bref.) (Aphyllophorales, Polyporaceae). The disease is widespread in coniferous forests of the Northern Hemisphere and is a major causes of tree mortality in North America (Goheen and Goheen 1989; Slaughter and Parmeter 1989; Smith 1984; Wallis and Reynolds 1970). In North America, *H. annosum* includes the S and P intersterility groups (ISGs) (Chase 1989). *Abies* spp., *Pseudotsuga*, *Picea* spp., *Tsuga* spp., and *Sequoia* spp. are infected by S ISG isolates. *Pinus* spp., *Juniperus* spp., and *Calocedrus* are most commonly infected by the P ISG isolates (Chase 1989; Otrosina and Cobb 1989), but can occasionally be infected by S ISG isolates (Garbelotto et al. 1996).

Similar to other root diseases, e.g., blackstain disease (causal agent = *Leptographium wagenerii* (Kendr.) Wingf.), laminated root rot (causal agent = *Phellinus weirii* (Murr.) Gilb.) and *Armillaria* root rot (causal agent = *Armillaria* spp.), tree mortality appears in clusters that enlarge gradually (Bloomberg and Reynolds 1982; Childs 1963; Cobb et al. 1982; Shaw 1980; Wallis and Reynolds 1965; Worrall 1994). Unlike other root pathogens, however, *H. annosum* is incapable of growing freely in the soil (Hodges 1969), and there is no documentation of significant insect vectors (Hunt and Cobb 1982; Nuorteva and Laine 1968). Tree-to-tree transmission is most likely by secondary growth of the fungus inside grafted roots, or by ectotrophic growth of fungal hyphae on the bark of roots in contact with each other (Hodges 1969; Rishbeth 1950).

Secondary spread has been documented in pine plantations (Hodges 1974; Rishbeth 1951), Norway spruce (*Picea abies* (L.) Karst.) stands (Stenlid 1985), Sitka spruce (*Picea sitchensis* (Bong.) Carrière) plantations (Morrison and Redfern 1994), ponderosa pine (*Pinus ponderosa* Dougl. ex P. & C. Laws.) and Jeffrey pine (*Pinus jeffreyi* E. Murr.) stands (Ratcliff et al. 1993), and white fir (*Abies concolor* (Gord. & Glend.) Lindl.) mortality centers in mixed-conifer stands (Garbelotto et al. 1993b). Freshly cut stumps are primary infection sites for basidiospores in pine and spruce (Hunt et al. 1976; Morrison and Johnson 1978; Morrison et al. 1986; Rishbeth 1951). Inoculation experiments have been performed on several
species, including pine and Sitka spruce stumps (Morrison and Redfern 1994; Rishbeth 1951), and the rate of spread of introduced isolates has been monitored.

The behavior of *H. annosum* in white fir mortality centers is different from that in pine or spruce centers (Otrosina and Cobb 1989), and is less studied. True firs are more likely to be infected via wounding than from nearby infected stumps (Otrosina and Cobb 1989; Garbelotto et al. 1993b). Secondary spread between infected stumps and neighboring trees is less frequent in white fir than in pine (Garbelotto et al. 1993b). Reduced virulence of isolates, tree and stand characteristics, or a combination of both may be responsible for the low incidence of stump to tree spread (Slaughter et al. 1991). Secondary spread among fir trees occurs when trees are closely spaced (Garbelotto et al. 1993b), but many aspects of the dynamics of expansion and arrest of the pathogen in true fir mortality centers are still unresolved.

Factors influencing the spread of annosus root disease include edaphic characteristics (Alexander et. al. 1975; Hodges 1969; Morris and Frazier 1966; Rishbeth 1951), previous silvicultural treatments (Filip 1993; Shaw at. al. 1993), and effects of ozone (James et al. 1980). More information is needed to understand modes of fungal infection and expansion, including pathogen-related, host-related and stand-level characters. Understanding the dynamics of secondary spread is pivotal to the development of adequate site risk-rating guidelines (Morris and Frazier 1966), and of models that may predict mortality caused by *H. annosum* in North American forests (Filip 1989; McNamee et al. 1991).

In a field inoculation experiment, roots of white fir were colonized when wood dowels infested with *H. annosum* were inserted into holes drilled in the root xylem (Garbelotto et al. 1997). Wood colonization rates matched fungal expansion rates observed in the field, and no differences were observed between homokaryotic (*n* genome) and heterokaryotic (*n* + *n* genome) isolates. The analysis of that data set is expanded in this study to include other factors that may regulate the colonization of *H. annosum* in live roots of white fir.

Lack of mortality surrounding stumps colonized by *H. annosum* (Slaughter and Parmeter 1995) may be determined by intrinsic factors (e.g., reduced pathogenic ability of saprobic stump isolates) or extrinsic factors (e.g., stand density, frequency of root contacts, presence of antagonistic microorganisms). We hypothesized (H1) that if intrinsic factors are relevant, isolates from stumps would be less efficient colonizers of live roots than isolates from live trees.

Root size may be a significant extrinsic factor regulating endotrophic root colonization. The cambium may have a negative effect on the growth of *H. annosum* in small roots of white fir, as shown for decay fungi in *Abies balsamea* (L.) Mill. (Worrall and Harrington 1988) and for *Phellinus weirii* in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Bloomberg and Reynolds 1982). Furthermore, antagonists may be harder to circumvent in smaller diameter roots than in larger ones. We hypothesized (H2) that *H. annosum* colonization is less in small roots than in larger roots.

Patterns in the development of mortality centers are hard to predict (Slaughter et al. 1991) and may be determined by differential root colonization in different directions, i.e., fungal colonization will proceed at a faster rate towards the tree (proximal direction) than away from it (distal direction), or vice versa. We hypothesized (H3) that fungal colonization would be greater towards the bole than away from it, because of the effects of cavitation in sapwood water columns under tension.

**Materials and methods**

**Fungal isolates**

Eight isolates were used in the inoculation study. All isolates were collected from trees or stumps of white fir in California. Somatic compatibility tests (Stenlid 1985) indicated that all isolates were incompatible with one another, and thus represented eight different genotypes. All were mycelial isolates obtained from wood. Each of four “tree” isolates was obtained from a different white fir mortality center in the Eldorado National Forest in the Sierra Nevada. Each tree isolate was present in at least two symptomatic trees (Garbelotto et al. 1993b). That these genotypes had spread to at least two trees was considered a sign of their virulence. Tree isolates were homokaryotic. The remaining four isolates were collected from three stumps at three locations in the Lassen National Forest. Two isolates were obtained from the same stump. Stump isolates were heterokaryotic, had extensively colonized their hosts, and had fruited abundantly. The degree of wood decay indicated that the isolates had been actively growing in these stumps for at least 10–15 years. These isolates may be less pathogenic than the tree isolates, because no mortality had occurred in proximity to the stumps in the previous 10–15 years. Random amplified polymorphic DNAs (RAPDs) were used to determine the intersterility group of the isolates (Garbelotto et al. 1993a). All eight isolates belonged to the S ISG.

Isolates were grown and stored on standard malt extract agar medium (MEA) in 9-cm Petri dishes. A set of cultures was kept at 4°C. For inoculation purposes, the isolates were grown for 12 weeks on pine wood dowels (approximately 0.6 cm diameter and 3 cm length) previously immersed in malt extract broth for 2 h and then placed on MEA plates.

**Study site**

The experiment site (240 × 150 m, slope 0–10%) was located at an elevation of 1650 m in the Mi-Wok district of the Stanislaus National Forest (38°04′21″N, 120°01′16″W). White fir was the dominant species in the stand (average height = 40 m), but ponderosa and sugar pine (*Pinus lambertiana* Doug.) as well as incense cedar (*Calocedrus decurrens* (Torr.) Florin) and Douglas-fir were present. White fir regeneration also was present in stand openings. Increment boring at breast height of three randomly selected trees indicated that the stand was approximately 95 to 105 years old. No obvious disease signs or symptoms were observed, although adjacent stands had stumps colonized by *H. annosum*.

Fifty dominant or codominant and apparently healthy trees were selected for inoculation. Crown ratios (percent of tree height occupied by live crown) ranged between 30 and 60%, and mean DBH was 94 cm (SD = 13.4). Forty-eight trees were divided into eight blocks made up of six adjacent trees. The remaining two trees (indicator trees) were inoculated and sampled frequently to assess fungal growth. Rhizomorphs of *Ammarias* spp. sometimes were encountered on the roots to be inoculated, but no associated internal decay was evident.

**Inoculation, sampling, and genotypic identification of fungal isolates**

Inoculations were performed at the beginning of June 1993, just before the beginning of the vegetative growth of white fir. Two hundred roots (four per tree) with diameters larger than 4 cm were inoculated with wood dowels colonized by *H. annosum* isolates. One root served as a control and was inoculated with a sterile wood dowel. One tree isolate and two stump isolates were selected and used to inoculate...
three separate roots of each tree. Although only three isolates could be inoculated onto a single tree, all eight fungal isolates were represented in each block (18 roots in six trees) and randomly assigned to a root.

Inoculation and sampling of tree roots were performed as described in Garbelotto et al. (1997) and are summarized here. Fungus-colonized wood dawels were inserted into holes drilled in the roots 75–100 cm from the root collar. Based on the extensive fungal colonization observed in roots of indicator trees 3 months postinoculation, all of the trees in two of the eight experimental blocks were sampled 4 months postinoculation. All of the trees in the remaining six blocks were sampled 12 months postinoculation. Inoculated roots were sampled by excavating the roots to the root collar (proximal direction) and at least 1 m beyond the inoculation point (distal direction). Roots then were removed from the tree with a chainsaw, and the ends were covered with plastic bags.

Each root was transversally sectioned into 5 cm thick disks with a chainsaw. The chainsaw bar was immersed in a 10% household bleach solution in between each cut. Each disk was labeled and placed in a separate plastic bag. Disks were incubated for 7–10 days at room temperature and then were microscopically observed for the presence of the imperfect stage of *H. annosum* (*Spiniger meineckellus* (Olson) Stalpers). The relative distance of each disk from the inoculation point was recorded; thus, the number of disks colonized by *H. annosum* was assumed to represent the extent of fungal growth in the inoculated roots.

Wood chips from root disks colonized by *H. annosum* were plated on MEA, and pure fungal cultures were obtained. Somatic compatibility tests were performed to verify that a single fungal genotype per root was present, and to confirm that the isolate was the same one inoculated into the root (Stenlid 1985).

**Measurements**

Diameters of proximal and distal root portions were taken as the average of the largest and the smallest diameter of the median section in the portion. Distal and proximal portions of roots were assigned to three classes according to their diameters: class I included 46 roots with distal diameters ranging from 4 to 9 cm; class II included 50 roots with distal diameters ranging from 10 to 15 cm; class III included 40 roots with distal diameters ranging from 16 to 36 cm.

Fungal colonization was recorded in increments of 2.5 cm because roots were sectioned into 5 cm thick disks (longitudinal colonization). When the proximal side of a disk was positive for *H. annosum* and the distal side was negative, it was assumed that the fungus had colonized half of the disk (i.e., 2.5 cm).

The percentage of root area occupied by *H. annosum* associated stain or incipient decay (radial colonization) was calculated for three sections per each root (inoculation section, farthest distal, and farthest proximal sections).

**Statistical analyses**

All statistical analyses were performed using the statistical program “Statgraphics” (Statistical Graphics Corporation 1987, Rockville, Md.). Statistical analyses presented here did not include data from unsuccessful inoculations because analyses including these data did not change the interpretation of the results (data not shown).

Bartlett’s and Cochran’s tests were employed to verify the homogeneity of variances; \( \chi^2 \) and Kolmogorov’s tests were used to test for normality of the data. The data fit the requirements of normality and homogeneity of variances necessary to employ standard parametric tests such as ANOVA and Student’s \( t \)-test. ANOVA was used to compare fungal colonization among inoculation blocks. Because no statistically significant differences were found among blocks or between distal colonization after 4 and 12 months, the statistical analysis of distal colonization was performed, including all roots according to the formula for a completely randomized test. Analysis of proximal colonization was performed separately for blocks sampled after 4 and 12 months. ANOVA was used to compare (a) length of colonized roots and percentage of root cross section discolored by isolates from stumps versus isolates from trees, (b) radial and longitudinal colonization of fungal isolates in roots of three diameter classes. The Tukey test was employed for multiple range analyses. Student’s \( t \)-tests were used to compare radial and longitudinal fungal colonization after 4 and 12 months.

**Results**

**Inoculation, sampling, and measurements**

A total of 190 roots were sampled, including eight from the two indicator trees. Ten inoculated roots could not be located for sampling. Fungal colonization was not detected in any of the 48 control roots. Fungal colonization was detected in 111 (83%) of 134 inoculated roots. The fungus had colonized the root xylem, but was not isolated from the phloem or bark. Positive results from somatic compatibility tests confirmed that fungal isolates reisolated from 105 of 111 colonized roots were the same as those used as inoculum. The remaining six isolates could not be matched to the inoculum isolates and were not considered in the analyses.

In 67 (50%) inoculated roots, the fungus had grown from the roots into the bole. In these cases, accurate measurement of fungal growth was not possible. The proportion of inoculations that resulted in infection of the bole was the same for all eight isolates. In five (3%) inoculations, the fungus had outgrown the sampled distal portion of the root. Therefore, total distal growth was determined accurately. Comparative analyses of colonization among isolates from trees and stumps were performed both excluding and including measurements of isolates that grew into tree boles. Interpretation of the results was identical in both cases. Therefore, root colonization that had progressed to the bole was included in comparative analyses of fungal colonization rates in the proximal and distal directions, in the analyses comparing fungal colonization after 4 and 12 months, and in the analysis of colonization values in different diameter-class roots.

Wood colonized by *H. annosum* always displayed a purple-brown discoloration, which often occurred beyond the boundaries of fungal colonization. When present, conidiophores always grew within the discolored area.

**Longitudinal fungal colonization**

**Fungal growth at 4 versus 12 months:** Vegetative growth of *H. annosum* occurred in both the distal and proximal directions. In the distal direction, growth occurred primarily during the summer and early fall (Table 1) because distal colonization after 4 and 12 months did not differ significantly (\( \alpha = 0.05, P = 0.44, df = 105 \)). However, proximal colonization after 12 months was significantly greater than colonization at 4 months (\( P < 0.001, df = 106, \alpha = 0.05 \)), indicating that fungal isolates had continued to colonize roots in the proximal direction during the winter and spring months (Table 1).

**Isolates from trees and stumps:** Because isolates from stumps were heterokaryons and isolates from trees were homokaryons, the comparative analysis of tree versus stump isolates was identical with that of homokaryons versus heterokaryons presented in Garbelotto et al. (1997). However, results presented here differ from those of Garbelotto et al.

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significant differences were not observed (P = 0.054). At 12 months, colonization in the proximal direction was significantly greater than that in the distal direction (P < 0.001, df = 149) (Table 1). To eliminate the effect of small-diameter roots on distal fungal colonization values, a t-test comparing proximal and distal colonization was performed excluding values from roots belonging to the first diameter class (root diameter less than or equal to 9 cm). The difference between proximal and distal fungal colonization was still significant (P < 0.001, df = 104).

**Radial fungal colonization**

Stain caused by *H. annosum* was large enough to be measured in 111 inoculated roots. Percentages of root transversal area occupied by the stain averaged 30% at the inoculation point, 28% at the last colonized proximal section, and 26% at the last colonized distal section. ANOVA (data not shown) detected no differences in percentages of wood discoloration associated with any of the eight fungus isolates, at any of the three sections. A t-test (not shown) indicated that percentages of discoloration were not different in the proximal and distal directions.

At the inoculation point, the percentage of root cross sections occupied by the stain averaged 25% (SD = 14) at 4 months postinoculation, and 32% (SD = 16) at 12 months postinoculation (t = -2.45, df = 110, α = 0.05, P = 0.016). The percentages of sections with stain at the inoculation point differed significantly among the three root diameter classes at 4 months, but not at 12 months (Table 4). At 4 months, in fact, the average percentage of stained cross sections in roots belonging to the largest diameter class was significantly less than

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Table 1. ANOVA of proximal and distal average colonization of white fir roots by eight *H. annosum* isolates 4 and 12 months postinoculation.

<table>
<thead>
<tr>
<th></th>
<th>Proximal colonization (cm)*</th>
<th>Distal colonization (cm)*</th>
<th>t</th>
<th>df</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 months</td>
<td>36 (2.9)</td>
<td>28 (2.4)</td>
<td>-1.96</td>
<td>62</td>
<td>0.054</td>
</tr>
<tr>
<td>12 months</td>
<td>49 (2)</td>
<td>31 (2)</td>
<td>-6.28</td>
<td>149</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t</td>
<td>-3.63</td>
<td>-0.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>106</td>
<td>105</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P*</td>
<td>&lt;0.001</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Values in parentheses are standard errors. Student’s t-tests were used to compare colonization in the same direction of different times (columns), and colonization in different directions at the same time (rows).

*Measurements of isolates that grew into the boles are included; unsuccessful inoculations (i.e., no growth was measured) are excluded.

†α = 0.05.

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Table 2. ANOVA of distal and proximal growth of *H. annosum* isolates from trees and stumps.

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Distal growth* (4 and 12 months)</th>
<th>Proximal growth*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots (n)</td>
<td>Mean (cm)</td>
</tr>
<tr>
<td>From trees (n=4)</td>
<td>35</td>
<td>27.5 (2.8)</td>
</tr>
<tr>
<td>From stumps (n=4)</td>
<td>72</td>
<td>31 (1.9)</td>
</tr>
<tr>
<td>df</td>
<td>106</td>
<td>30</td>
</tr>
<tr>
<td>F-ratio</td>
<td>1.3</td>
<td>0.002</td>
</tr>
<tr>
<td>P</td>
<td>0.25</td>
<td>0.97</td>
</tr>
</tbody>
</table>

**Note:** Standard errors are given in parentheses.

*Measurements of isolates that grew into the boles are included; failed inoculations (i.e., no growth was measured) are excluded. Two different analyses (at 4 and 12 months) were performed for proximal growth data; see Table 1.
the averages in the other two classes. At 12 months root sections in all diameter classes were equally stained. Student’s t-tests were conducted to compare the percentages of stain at 4 and 12 months for root sections in each root diameter class: the only significant increase in percentage of stained root sections was in the largest diameter class (Table 4).

## Discussion

The inoculation technique was successful, as 83% of the inoculated roots became infected by *H. annosum*. Somatic compatibility tests confirmed in 95% of cases that the isolates recovered from inoculated roots were the same ones that had been inoculated. The inoculation technique mimicked secondary spread of the pathogen through root grafts. Inoculum dowels were similar to roots colonized by *H. annosum* grafted to healthy, uncolonized roots. The wounds inflicted on the roots by use of the drill were relatively small, and judging from the appearance of the mock inoculations, did not cause major physiological disturbance to the roots.

All fungal isolates used as inoculum were virulent as assessed by extent of wood colonization and staining (Tables 2 and 4). Average values of longitudinal colonization ranged from 23 to 58 cm, and maximum and minimum values ranged from 2.5 to 80 cm, and are similar to those (0.2–1 m) observed in a previous field study of mortality in white fir trees (Garbelotto et al. 1993b; M. Garbelotto, unpublished data). Reported colonization distances in other hosts ranged from 25 cm to 2.1 m per year in pine spp. (Hodges 1969, 1974; Hunt et al. 1976; James et al. 1980; Rishbeth 1950; Slaughter and Parmeter 1995), 12 to 31 cm per year in Norway spruce (Hodges 1969; Stenlid and Johansson 1987), and 5.5 to 32 cm per year in Sitka spruce (Hodges 1969; Morrison and Redfern 1994).

*Heterobasidion annosum* may lead to rapid loss of function in colonized xylem of white fir. In some cases, softness of the colonized xylem suggested that discoloration represents incipient decay. The lack of pathological discoloration in mock-inoculated roots indicated that the phenomenon was induced by *H. annosum* and not by wounding or contaminants. While *H. annosum* was always associated with discolored wood, pathological staining often occupied a larger portion of the root than that colonized by the pathogen. Similar observations have been reported in Norway spruce (Shain 1971, 1979; Stenlid and Johansson 1987). In spruce, stain was attributed to an increase in the local concentration of phenolic extractives.

No significant differences in colonization and pathological wood staining were found among these eight isolates (Garbelotto et al. 1997). Because homokaryotic isolates had been obtained from trees, and heterokaryotic isolates from stumps, the comparative analysis of root colonization by stump versus tree isolates yielded results identical with those obtained when comparing homokaryons with heterokaryons (Garbelotto et al. 1997) Similar amounts of fungal root colonization and stain were observed among all isolates regardless of their origin or nuclear condition.

Lack of spread of annosus root disease from stumps to neighboring trees has been reported (Slaughter et al. 1991). Root transfer between inoculated Sitka spruce stumps and neighboring trees occurred only at 22% of the viable root contacts (Morrison and Redfern 1994). Secondary spread may occur

### Table 3. ANOVA of proximal and distal colonization of white fir roots of three diameter classes by *H. annosum*.

<table>
<thead>
<tr>
<th>Root class</th>
<th>Diam. (cm)*</th>
<th>Root (n)</th>
<th>Mean growth (cm)†</th>
<th>Homogeneous groups‡</th>
<th>Root (n)</th>
<th>Mean growth (cm)†</th>
<th>Homogeneous groups‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4–9</td>
<td>46</td>
<td>20.5 (2.6)</td>
<td>a</td>
<td>12</td>
<td>37 (5)</td>
<td>a</td>
</tr>
<tr>
<td>II</td>
<td>10–15</td>
<td>50</td>
<td>34 (2.3)</td>
<td>b</td>
<td>15</td>
<td>47 (4.5)</td>
<td>ab</td>
</tr>
<tr>
<td>III</td>
<td>16–36</td>
<td>40</td>
<td>35 (2.7)</td>
<td>b</td>
<td>50</td>
<td>53 (2.5)</td>
<td>b</td>
</tr>
</tbody>
</table>

| F-ratio    | 12.7        | 4.26     |
| df         | 106         | 76      |
| P          | <0.001      | 0.017   |

Note: Standard errors are given in parentheses.

*Mean diameter of the median section of distal or proximal root portions colonized by the pathogen.

†Failed inoculations (i.e., no fungal growth was measured) were excluded; measurements from isolates that had grown into the boles were included.

‡Homogeneous groups were determined by Tukey’s multiple range tests.

Fig. 1. Linear regression analysis of the average distal growth of *H. annosum* in inoculated white fir roots versus root diameter. Results from a simple linear regression analysis are shown. The high $R^2$ value (0.89) indicates a significant correlation between fungal growth and root diameter; the linear equation $Y = 16.1 + 2.42X$ indicates that the correlation between the two variables is positive.
in white fir mortality centers, but it is infrequent and seems to occur only when trees are less than 6 m apart (Garbelotto et al. 1993b; M. Garbelotto, unpublished data). Our data suggest that lack of spread may not be caused by reduced virulence of fungal isolates. Alternatively, factors such as frequency of root contacts and (or) grafts, root size and architecture, and edaphic conditions may affect the efficiency of fungal spread.

Root diameter classes were selected based on empirical observations of root size at various Sierra Nevada sites. Most root grafts and root contacts in a mature white fir stand fall into the first diameter class (4–9 cm). Root contacts or grafts of the second class (10–15 cm) occur in trees less than 5–6 m apart. Only trees adjacent to each other (less than 1 m apart) have root contacts in the third class (16–36 cm). Smaller diameter roots (class I) were significantly less colonized by H. annosum than larger diameter roots (classes II and III). Fungus colonization may be less in small roots because of the inhibiting action of the phloem, the inability to circumvent antagonistic microorganisms, or the enhanced influence of environmental variables on the fungus. Large roots may be required for efficient movement of the pathogen along the root system. A sufficiently large root size at root contact or grafting points may be important to fungal spread from tree to tree or from stump to tree.

Average radial expansion of the fungus in the roots proceeds until about one-third of the root cross section is discolored. This expansion required a shorter time in smaller (4 months) than in larger roots (12 months). These results suggest that only a small amount of the root cross-sectional area is available for fungal colonization in living trees.

Ectotrophic mycelium was not evident in this and another study (Garbelotto et al. 1993b). Soils at sites in both studies were acidic. Low pH inhibits the formation of ectotrophic mycelium of H. annosum on the surface of pine roots (Rishbeth 1950). Ectotrophic mycelium may increase disease spread by allowing the pathogen to bypass roots that are damaged or colonized by other microorganisms. Without ectotrophic mycelium, the fungus must spread via endotrophic mycelium, which is limited by smaller root size. Limited fungal endotrophic growth in smaller roots has been observed also in the Douglas-fir – Phellinus weirii pathosystem (Bloomberg and Reynolds 1982).

Fungal growth in the proximal direction was significantly greater than growth in the distal direction; i.e., the pathogen grew faster from the inoculation point towards the bole than away from it. In addition, distal colonization may have occurred only during the vegetative growth season, while proximal spread continued into the dormant season. In roots of Abies sachalinensis (Mast.) infected with H. annosum, the fungus grew faster in the proximal than in the distal direction. Distal colonization values were 10–60 cm (Yokota 1962). These results and ours contrast with those from inoculation tests on pine (James et al. 1980; Wallis 1981). Differences in inoculation techniques, hosts, and fungal ISGs may account for the conflicting results. In true firs, H. annosum decays the sapwood and causes heartrot in living trees. When sapwood water columns are under tension (e.g., at the beginning of the vegetative growth season), xylem wounding will cause a withdrawal of water and the entry of air in a tapered region surrounding the wound (Rayner and Boddy 1988). As water flows from roots towards the bole, air movement also will be greater in the proximal than in the distal direction. Because decay fungi are obligate aerobes and their growth is stimulated by oxygen, this phenomenon may be partially responsible for accelerating fungal growth towards the root collar and the bole.

A practical implication of this observation is that radial expansion of the pathogen from infection foci will not be regular, but will proceed in waves, accelerating when new roots are infected, and continuing until the main stem is reached (proximal direction). The fungus then will colonize the lower portion of the bole and new roots (distal direction) at a rate slower than that of colonization in the proximal direction. According to this model, growth patterns of H. annosum would be asymmetric, with the asymmetry being directly related to the spacing among trees. The concept of differential growth rates of H. annosum has been suggested by Platt et al. (1965), who experimentally showed that root wood of several conifer species was more susceptible to colonization by H. annosum isolates than stemwood. This phenomenon was ascribed to the higher nitrogen content and reserve carbohydrates in root compared with stem wood. Thus, fungal hyphae moving from tree to tree should slow their spread whenever a tree stem is invaded.

The results presented here provide forest managers and root disease modelers with new information on rates and modes of

Table 4. Statistical analyses of radial stain caused by eight H. annosum isolates in white fir roots, at the inoculation point.

<table>
<thead>
<tr>
<th>Root class</th>
<th>Diam. (cm)</th>
<th>Roots (n)</th>
<th>Stain (%)*</th>
<th>Homogeneous groups†</th>
<th>df</th>
<th>t</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4–9</td>
<td>12</td>
<td>30 (3.2)</td>
<td>a</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>10–15</td>
<td>13</td>
<td>27 (3.7)</td>
<td>a</td>
<td>29</td>
<td>33 (3)</td>
<td>40</td>
</tr>
<tr>
<td>III</td>
<td>16–36</td>
<td>7</td>
<td>11 (5.2)</td>
<td>b</td>
<td>25</td>
<td>30 (3)</td>
<td>40</td>
</tr>
</tbody>
</table>

Note: Standard errors are given in parentheses. ANOVA was used to compare stain in different root diameter classes (columns); t-tests were used to compare stain levels at different times for each diameter class (rows).

*Area of stain/area of root section.
†Homogeneous groups according to Tukey’s multiple range test.
‡The results presented here provide forest managers and root disease modelers with new information on rates and modes of

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spread of *H. annosum* in white fir mortality centers in California. Based on comparisons of colonization values among eight isolates, we reject the hypothesis (H1) that lower virulence or uniquely saprobic isolates (intrinsic factors) are responsible for the lack of mortality around stumps colonized by *H. annosum*. Based on the reduced growth in small-diameter roots, and on the limitations in radial growth determined by root size, we accept the hypothesis (H2) that extrinsic factors, including small-diameter roots, inhibit the endotrophic growth of *H. annosum*. We also accept the hypothesis (H3) that fungal colonization will be greater in the proximal (towards the bole) than in the distal (away from the bole) direction.

Because tree root architecture is relevant to the spread of *H. annosum*, disease modelers and forest pathologists have to consider all factors that may determine such architecture, including genetic traits of the host plant, soil structure, composition of the plant community, and edaphic conditions. Rates of fungal colonization in roots are influenced also by the direction of the spread. Thus, more precise mortality predictions may be obtained by considering the location and size of host plants in an infested stand, and by mapping infection foci in relation to host distribution.

**References**


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