Spread of *Heterobasidion annosum* in Christmas Tree Plantations of the United States Pacific Northwest

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ABSTRACT

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The population structure of *Heterobasidion annosum* in the Pacific Northwest (PNW) Christmas tree plantations was estimated at two spatial scales to assess the relative importance of primary and secondary infection, colonization, and spread of the pathogen. Ninety-three isolates from single trees in 27 discrete mortality pockets and 104 isolates from 12 individual root systems of noble and Fraser fir trees were sampled near Mossyrock, Washington. Isolates were genotyped using somatic compati-

bility assays and microsatellite markers to determine the spatial scale at which dispersal of single genotypes (genets) was occurring. All isolates sampled from different trees in discrete mortality pockets had distinct genotypes, whereas the root systems of single trees were dominated by one or two genotypes. These results suggest that infection of PNW Christmas trees results from frequent primary infection events of adjacent stumps and localized secondary spread within root systems rather than clonal spread of the pathogen between adjacent trees. We hypothesize that mortality pockets may be due to availability of infection courts and/or variation in inoculum levels during selective harvesting of patches of mature trees.

The Pacific Northwest (PNW) leads the United States in production of Christmas trees. In 2005, approximately 40% of trees harvested in the United States came from the PNW and were worth an estimated 190 million dollars. As the PNW Christmas tree industry has become increasingly dependent on true firs species such as noble fir (*Abies procera* Rehd.), the incidence of annosus root rot disease in western Washington and western Oregon Christmas tree plantations has increased from less than 4% in 1980 to 24% in 2000. We have observed up to 15 and 40% mortality in severely infected noble and Fraser fir (*A. fraseri* (Pursh) Poir.) plantings (G. A. Chastagner, *unpublished data*), respectively, and this increase is thought to be due to a shift in the tree species being planted. To meet market demands for trees with better needle retention and aesthetic qualities, growers began to replace Douglas-fir plantings with true firs in the late 1970s and early 1980s (1,2).

Annosus root rot is caused by *Heterobasidion annosum* (Fr.) Bref. and this organism is considered an economically important pathogen of trees in the majority of the northern temperate forests of Europe and North America (9). Infected stems develop butt rot and heartwood staining, rendering some trees unmarketable and significantly reducing the value of others. Infection of roots results in reduced growth rates and premature death (7). In the PNW, *H. annosum* infects a diversity of native conifers such as grand fir (*A. grandis* (Dougl. Ex D. Don) Lindl.), noble fir, white fir (*A. concolor* (Gord. & Glend.) Lindl. Ex Hildebr.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). The susceptibility of these species to annosus root rot varies widely. For example, Douglas-fir is resistant, whereas grand fir, noble fir, white fir, and western hemlock are susceptible (7). In stands of noble fir and western

hemlock in coastal Oregon and Washington, *H. annosus* infects up to 90% of western hemlock (3) and 75% of noble fir in previously thinned stands (25).

In North America, the name *H. annosum* refers to a species complex consisting of two intersterility groups (ISGs) known as S and P strains that have different host specificities (28). S and P strains are generally found associated with spruce (*Picea* spp.), and pine (*Pinus* spp.), respectively (10), and both strains are found in Pacific coastal forests of North America (6). The S strain has been isolated from true firs, giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchholz), pines, western red cedar (*Thuja plicata* Donn ex D. Don), and western juniper (*Juniperus occidentalis* Hook). In contrast, the P strain has been isolated from various pine species, western red cedar, and western juniper (5,6). Although no comprehensive study of the distribution of S and P strains has been conducted in Washington or Oregon, only S-strain *H. annosum* has been recovered from western hemlock, white fir, and noble fir in PNW forests (4,5,25). The identity of *H. annosum* strains infecting Christmas trees in PNW plantations has not yet been determined.

Primary infection of *H. annosum* occurs when monokaryotic basidiospores or conidia land and germinate on freshly cut stumps or wounded stems (17-19,23). The fungus colonizes the heartwood and sapwood and monokaryotic (n) hyphae fuse to form dikaryotic (n+n) mycelium when compatible hyphae contact one another (9). The dikaryotic form of the fungus continues to spread secondarily to lateral roots and adjacent trees through root contacts which can result in expanding mortality pockets as has been observed in forest settings (5,13,17,22,24,27). Evidence of nuclear sharing among adjacent genets of *H. annosum* isolated from primarily infected stumps and boles suggest that the dikaryotic state of *H. annosum* may not be stable and that dikaryon-monokaryon matings may be common in nature (5,8,9). In contrast, dikaryons of a single genotype can be found colonizing multiple trees in mortality pockets, suggesting that under certain conditions, the fungus may exist as a stable dikaryon (5,22).

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The extent of secondary infection and resultant size of genets occupying mortality pockets varies by host tree and site history. In long-established Norway spruce (*Picea abies* (L.) Karst.) stands in Sweden, single genets of the pathogen have been found to colonize up to 13 adjacent trees (22). Disease foci in a native selectively managed Californian white fir (*A. concolor* (Gord. & Glend.) Lindl. ex Hildebr.) forest have been found to contain several genets, with a single genet infecting as many as nine adjacent trees (5). Although vegetative spread between root contact is commonly reported in mature, less intensively managed forest settings, genets are confined to single trees in more intensively managed, first rotation plantings (26). In PNW Christmas tree plantations, nonrandom aggregations of diseased trees (mortality pockets) have been observed which appear similar to clusters of infected trees observed in forest situations. Stump removal practiced by PNW Christmas tree growers has greatly reduced annosus root rot in Christmas tree plantations, indicating the importance of stumps as primary infection courts (G. A. Chastagner, *unpublished data*). These mortality pockets could result from clonal spread of the pathogen among adjacent trees following infection of a single stump or multiple, spatially clustered stump infections by different genotypes of the pathogen. Root systems of adjacent Christmas trees are often connected below the soil surface (G. A. Chastagner, *unpublished data*), which would allow tree-to-tree spread of the pathogen and result in the same genet occupying groups of adjacent symptomatic trees. To differentiate between these two hypotheses, we sampled *H. annosum* isolates at different ecological scales and genotyped them using micro-satellite markers and ISG phenotyping. Our specific objectives were (i) to identify strains of *H. annosum* causing annosus root rot of Christmas trees in the PNW and (ii) to estimate genotypic diversity of the pathogen at the mortality pocket scale and the individual root system scale. These data have allowed us to determine the importance of primary infection versus secondary spread of annosus root rot disease in Christmas tree plantations and will aid in development of improved annosus root rot management strategies.

MATERIALS AND METHODS

Regional scale sampling. To identify strains of *H. annosum* infecting Christmas trees in the PNW, 63 isolates were sampled during the summers of 2001 and 2002 from five hosts in four Christmas tree plantations located near Orting, Satsup, and Mossyrock, Washington, and Hillsboro, Oregon (Fig. 1). One isolate was sampled per tree. Hosts included Fraser fir, grand fir, noble fir, Nordmann fir (*A. nordmanniana* (Steven (Spach)), and

Fig. 1. Locations of Christmas tree plantations from which isolates of *Heterobasidion annosum* were sampled for strain identification. Intensive sampling of isolates was performed at the Mossyrock site for mortality pocket and root system-level genotypic diversity analysis.

Douglas-fir that were 2 to 5 years old. Wood pieces were checked every 2 to 3 days for sporulating conidiophores of *H. annosum*. When asexual fruiting structures were observed, a single clump of conidia was transferred to *H. annosum* selective *H. annosum*-selective medium (11), grown for 1 week, and transferred to potato dextrose agar or malt extract agar (MEA) slants for long-term storage at 4°C.

Mortality pocket scale sampling. For genotypic diversity assessment at the mortality pocket scale, symptomatic trees in mortality pockets in three fields at the Mossyrock plantation were mapped and *H. annosum* sampled as described above. One of the fields, designated TF3, was a monoculture of noble fir consisting of 1,045 trees. The other two fields, designated TF2 and TF5, were Fraser fir monocultures consisting of 910 trees each. Within 3 to 6 years of replanting these fields, percent mortality due to annosus root rot had reached 10 to 40% within these fields. Ninety-three *H. annosum* isolates were obtained from single trees in 27 discrete mortality pockets of two to eight trees spread throughout the three fields (Fig. 2). Most of the sampled trees had been planted directly next to a stump left from the previous rotation, and all trees had been planted on a 1.8 by 1.8 m spacing pattern.

Root system scale sampling. Root systems were excavated from fields TF2, TF3, TF4, and TF5 within the Mossyrock plantation. These root systems were labeled RGS1 to RGS12. Eight of the root systems were from Fraser fir and four were from noble fir. To estimate number and distribution of *H. annosum* genotypes at the root system scale, root systems of the freshly killed trees were excavated and sampled using the isolation methods described above. *H. annosum* isolates were sampled from different

Fig. 2. Locations of mortality pockets (circled) within fields TF2 (top), TF3 (middle), and TF5 (bottom) at the Mossyrock plantation. Each dot represents a symptomatic tree from which a single isolate of *Heterobasidion annosum* was obtained.

parts of each excavated root system to obtain an estimate of the spatial distribution of genotypes at this scale.

DNA extraction. Isolates were grown at 18 to 24°C in 125-ml Erlenmeyer flasks containing 35 ml of 2.5% malt extract broth for 1 to 2 weeks on a rotary shaker (5). Mycelium was harvested by vacuum filtration and frozen at -20°C, and DNA was extracted with the BIO101 FastDNA Kit (Q-Biogene, Solon, OH) following the manufacturer's instructions.

ISG identification. Isolates were typed to strain using a polymerase chain reaction (PCR) assay referred to as taxon specific competitive priming (TSCP), which has been shown to be a robust proxy for ISG (6). TSCP-PCR (6) was performed with *H. annosum* ISG-specific primers S1 (5'-GCCGCGTCTCTCA-CAAAC) (160 nM/25- μ l reaction) and P1 (5'-GTCGGTCGGG-TCTTTTGATC) (0.16 μ M/25- μ l reaction) and universal primer pair ITS1 (0.16 μ M/25- μ l reaction) and ITS4 (0.5 μ M/25- μ l reaction). All reactions were carried out in 25- μ l volumes containing 10 mM Tris-HCl (pH 9.0), 50 mM KCL, 2 mM MgCl₂, 200 mM of each dNTP, 1 unit of *Taq* DNA polymerase (New England BioLabs Inc., Ipswich, MA), and 10 to 100 ng of template DNA. Amplification was performed with a Hybaid Omn-E thermocycler (Hybaid, Middlesex, UK) programmed for an initial cycle of denaturation at 95°C for 3 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. This initial cycle was followed by 30 cycles of 95°C for 30 s, 58°C for 1 min, 72°C for 2 min, and a final extension cycle of 5 min at 72°C. Controls for the TSCP-PCR consisted of template DNA of characterized S and P strain isolates (two to three of each) supplied by T. C. Harrington, Department of Plant Pathology, Iowa State University. PCR amplicons were separated in 3% agarose gels at 14 volts per cm for 1 to 2 h, stained with ethidium bromide, and visualized with a digital imaging system (BioDoc-It, UVP, Upland, CA).

Somatic incompatibility testing. A subset of three isolates from 10 of the 27 discrete mortality pockets, and all of the isolates sampled from single root systems were paired for somatic compatibility testing (22,25). Each pairing was replicated once. Plugs of MEA colonized by the mycelium of each isolate were placed on 2.5% MEA in 9 cm petri dishes, incubated at 18 to 21°C for 3 weeks in the dark, and then observed for zones of interaction between isolates. The morphology of the interaction zone was recorded as somatically compatible, which occurs between identical genets of *H. annosum*, or somatically incompatible, which occurs between genetically distinct isolates of *H. annosum* (22). Reactions were considered somatically compatible if the mycelium of isolates interacted freely and no demarcation line was apparent after 3 weeks. Reactions between paired isolates were considered somatically incompatible if a distinct demarcation line developed between isolates such as a gap or thin ridge of thick hyphal growth as described previously (22,25).

Microsatellite amplification, separation, scoring, and statistical analysis. Four microsatellite loci, designated Hams-1, Hams-2, Hams-6, and Hams-10 (9), were used to determine allelic and genotypic diversity of *H. annosum* isolates sampled at the mortality pocket and root system scales. Three microsatellite loci, Hams-1, Hams-6, and Hams-10, were used to genotype isolates sampled from single root systems. PCR was carried out in 25- μ l reaction volumes containing 1 $\times$ PCR buffer, 200 μ M of each dNTP, 10 pmol of both fluorescently labeled primers, 1 unit of *Taq* DNA polymerase (New England BioLabs Inc.), and 10 to 50 ng of template DNA. Reactions were performed in a Hybaid Omn-E thermocycler (Hybaid) set for an initial denaturation step of 97°C for 1 min, and then 35 cycles of 97°C for 30 s, 56°C for 30 s, and 72°C for 30 s, followed by a final extension step of 72°C for 7 min. Fragment sizes of PCR amplicons were determined with an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA). Alleles at each locus were visualized and scored individually to the nearest base pair using

GENESCAN and also interpreted with the aid of Genotyper version 3.7 (Applied Biosystems).

Numbers of alleles, molecular analysis of variance (AMOVA), and spatial autocorrelation analysis was performed using GenALEX version 5.1 (14). Input data for AMOVA and spatial autocorrelation analyses were in the form of a pair-wise multilocus distance matrix. Nonparametric statistical analyses were performed using 999 random permutations of the microsatellite allelic data (14).

RESULTS

ISG identification. All 63 isolates sampled from PNW Christmas tree plantations in Washington and Oregon were identified as S-strain *H. annosum* using TSCP-PCR. TSCP-PCR products of control P-strain and S-strain isolates were approximately 518 and 486 bp in length, respectively. All *H. annosum* isolates from PNW Christmas trees produced 486-bp amplicons (Fig. 3).

Population structure-mortality pocket scale. *Somatic compatibility tests.* Two types of interactions were observed when isolates were paired from the same mortality pocket: (i) somatically compatible interactions defined as a continuous mycelial mat occurred when isolates were paired with themselves, and (ii) somatically incompatible interactions occurred when each isolate was paired against any other isolate from the same mortality pocket. The morphology of the demarcation lines (width and sharpness) varied but were easily distinguishable from same-isolate, compatible pairings. From these data it was inferred that all isolates sampled from unique trees in mortality pockets were genetically distinct.

Microsatellite genotyping. Ninety-three multilocus genotypes were identified among 93 isolates sampled from unique trees forming mortality pockets at the Mossyrock plantation using four microsatellite markers. Number of alleles per locus ranged from 7 to 19. Sixteen alleles were observed at locus Hams-1, 7 alleles at locus Hams-2, 17 alleles at locus Hams-6, and 19 alleles at locus Hams-10. Most isolates had two alleles per locus at two or more loci indicating that they were likely heterokaryons. Approximately 12% (11 isolates) of the Mossyrock isolates had a single allele at all four loci, indicating that these isolates were likely homokaryons. Each mortality pocket contained an average of 3.61 isolates and 3.61 multilocus genotypes (Fig. 4). On average, there were 3.53 alleles per locus among all isolates within each discrete mortality pocket with a standard deviation (SD) of 0.62. The average frequency of least common alleles (alleles that occur in less than 50% of the isolates) was 2.23 alleles per pocket (SD = 0.62), and the frequency of private alleles (alleles exclusive to a mortality pocket) was 0.19 alleles per pocket (SD = 0.61). Within the 27 pockets the number of polymorphic alleles per pocket ranged from 50 to 100% with a mean of 89% polymorphic loci per pocket.

Fig. 3. A 3% agarose gel showing taxon specific competitive priming-polymerase chain reaction (PCR) products for 14 isolates of *Heterobasidion annosum*. Lanes 2 to 3 are PCR products of known S-strain isolates; lanes 4 to 6 are known P-strain isolates; and lanes 7 to 15 are isolates sampled from Pacific Northwest Christmas tree plantations.

Analysis of molecular variance (AMOVA) based on four microsatellite loci (12) indicated that a large (88%) and statistically significant ($P < 0.001$) portion of the genetic variability was observed among isolates within mortality pockets. AMOVA also indicated that small but statistically significant ($P < 0.001$) additional genetic variation occurred among isolates from different fields within plantations (9%), and among isolates grouped by mortality pocket within fields (3%) (Table 1). Spatial autocorrelation analysis (21) indicated no statistically significant spatial structuring of genotypes grouped in distance classes defined as trees equal or closer than 1, 4, 20, and 100 spacings away from each another.

Population structure–root system scale. Between 4 and 14 isolates were sampled throughout each of 12 root systems (RGS1 to RGS12), with an average of 8.42 isolates sampled per root system. The number of lateral roots isolated from within each root system ranged from two to five, with a mean of 3.42 lateral roots sampled per root system.

We recovered an average of 1.17 genotypes per root system compared with an average of 3.61 genotypes per mortality pocket (Fig. 5). DNA fingerprints generated for isolates from root systems RGS2, RGS3, RGS4, RGS9, RGS10, and RGS12 using microsatellite markers, demonstrated the occurrence of a single multilocus genotype in root systems RGS3, RGS4, RGS9, and

RGS10, and two genotypes in root systems RGS2 and RGS12. Somatic compatibility tests indicated a single genotype was isolated from 10 of the root systems (RGS1, RGS3, RGS4, RGS5, RGS6, RGS7, RGS8, RGS9, RGS10, and RGS11), and also differentiated two genotypes from cultures isolated from root systems RGS2 and RGS12.

The two genotypes isolated from root system RGS2 were designated ARGS2 and BRGS2, and the two genotypes isolated from root system RGS12 were designated ARGS12 and BRGS12. The distribution of genotypes within the RGS2 and RGS12 root systems is illustrated in Figure 4. The area occupied by genotypes ARGS2 and BRGS2 overlapped within the root system of RGS2 by approximately 20 cm. In contrast, the domains occupied by genotypes BRGS12 and ARGS12 in root system RGS12 did not overlap. In root system RGS12, genotype BRGS12 occupied all major lateral roots and ARGS12 was only isolated from the lower stem.

DISCUSSION

Annosus root rot in PNW Christmas tree plantations is caused by the S strain of *H. annosum* and this study confirms the presence of the S strain on true firs in both forest settings and Christmas tree plantations. These findings agree with studies conducted in stands of true fir in California and the PNW in which the S strain was the only strain found (4,5,25). Microsatellite fingerprinting and somatic compatibility testing demonstrated that individual trees within mortality pockets at the Mossyrock plantation were each colonized by a distinct genotype of *H. annosum*. The most obvious explanation for a nonrandom pattern of disease incidence in Christmas tree plantations (mortality pockets) would be secondary spread of a single genet from a single diseased tree. However, this is not what we found and our data allows us to reject the hypothesis of clonal spread among trees within mortality pockets. The high genotypic diversity observed within mortality pockets may indicate high levels of primary stump infection, combined with localized clonal spread within root systems, as seen in younger intensively managed forests (26). We hypothesize that mortality pockets result from availability of infection courts and/or variation in inoculum levels during selective harvesting of adjacent mature trees rather than clonal spread through root-to-root contact. This hypothesis is supported by anecdotal observations in the PNW Christmas tree plantations where trees are often harvested selectively in clusters as they reach merchantable size. It is thus possible that mortality pockets are the result of clusters of primarily infected stumps from which trees were harvested when high levels of inoculum were in the air, rather than the extensive spread of a single genet between trees via interconnected root systems. In order to test this hypothesis, it would be necessary to collect data on harvest date, map locations

Fig. 4. Distribution of *Heterobasidion annosum* genotypes within single root systems of true fir (*Abies* spp.) Christmas trees in which more than one genotype was isolated.

TABLE 1. Analysis of molecular variance among 93 *Heterobasidion annosum* isolates sampled from 27 mortality pockets in three fields

Source of variation	df	Sum of squares	Mean squares	Variance	Variance (%)	P value ^a
Among fields	2	10.577	5.289	0.044	9	<0.001
Among mortality pockets/ fields	24	63.967	2.665	0.208	3	<0.001
Among individuals/ mortality pockets	159	198.542	1.249	1.249	88	<0.001

^a P value is the probability of a larger variance component than observed by chance alone based on 999 random permutations of the entire sample without regard to the location of each isolate in original mortality pocket.

Fig. 5. Comparison of the mean number of genotypes of *Heterobasidion annosum* found at the root system and mortality pocket sampling scales as determined by DNA fingerprinting and somatic compatibility testing.

of harvested trees, and to relate these data to the subsequent spatial distribution of disease in the plantation.

No spatial aggregation of genotypes was observed at the mortality pocket level and alleles were randomly distributed throughout each mortality pocket at the Mossyrock plantation. On average, the least common alleles (alleles that occurred in less than 50% of the isolates) formed over one-third of the alleles sampled within each mortality pocket, indicating that low-frequency alleles were well distributed. The average number of private alleles (20) per pocket (alleles exclusive to a given mortality pocket) was relatively low, averaging 0.19 per mortality pocket which also suggests extensive migration of alleles among mortality pockets or founding from a common source population (20). These descriptive statistics all support the hypothesis that there is no genetic differentiation among mortality pockets and that alleles are randomly distributed.

To substantiate the observation of limited substructuring at the mortality pocket level, two statistical tests (12) showed a large (88%) and statistically significant ($P < 0.001$) portion of the genetic variability was observed among isolates sampled within mortality pockets and a much smaller percentage (3%) of variation was observed among isolates from different mortality pockets. Secondly, spatial autocorrelation analysis (21) indicated no statistically significant genetic structure among isolates grouped by distance class defined as trees equal or closer than 1, 4, 20, and 100 spacings away from one another. Assuming that genetic structure is an indicator of haploid basidiospore dispersal, these results suggest that basidiospores from genetically diverse sporocarps are randomly dispersed at the mortality pocket level. Furthermore, the data suggest genotypes composed of genetically diverse nuclei are infecting trees in close proximity (as close as one spacing, 1.8 m) through primary infection of local infection courts (such as closest adjacent stumps). Basidiocarps of *H. annosum* are rarely observed in even the most severely diseased PNW Christmas tree plantations, indicating that basidiospores are likely blown in from surrounding forests or that conidia may be functioning as primary inoculum.

Compared with the mortality pocket scale, genotypic diversity was low within root systems (Fig. 5). DNA fingerprinting and somatic compatibility tests both confirmed that 10 of 12 root systems were colonized by a single genotype, and two root systems were found to contain two genotypes. Low genotypic diversity of *H. annosum* within infected root systems has been previously reported with an average of one to two genotypes per root system (7,16,26). In contrast to root systems, infected stumps are often colonized by several genotypes (9,26). It has been suggested that intraspecific competition among genotypes, and the position of genotypes within newly colonized stump roots in relation to contacts with living trees results in lower genotypic diversity in secondarily infected trees (24,26). The observation of low genotypic diversity within secondarily infected root systems in Christmas tree plantations thus does not contradict the observation of high genotypic diversity among trees within mortality pockets. When combining these two results it supports the hypothesis that secondary spread is occurring repeatedly on a localized scale from primarily infected closest adjacent stumps by well established, competitively fit genotypes that have successfully out-competed other genotypes in primarily infected stumps. Johannesson and Stenlid (9) demonstrated that nuclear transfer among adjacent genets within primarily infected Norway spruce stumps may be common. They identified multiple nuclei that were shared among several adjacent, somatically incompatible isolates sampled from within a primarily infected stump using the same set of microsatellite markers that were used in this study. Garbelotto et al. (5) drew similar conclusions using arbitrarily primed-PCR fingerprinting to genotype isolates collected from stumps and boles of white fir trees in California. Upon careful inspection of our allelic data, we found that isolates collected

from adjacent Christmas trees generally shared less than 50% of alleles across all loci, which indicates a low probability of nuclear sharing between isolates infecting neighboring trees. We did not sample from primarily infected stumps in this study, but if we had done so, we may have been more likely to find isolates with shared nuclei (5,8,9). Interestingly, in the presumably secondarily infected root system RGS2, genotypes ARGS2 and BRGS2 overlapped in space within the root system and presumably had opportunity to interact (Fig. 4). These two genotypes, however, shared only a single allele at microsatellite locus Hams-1, which rules out the possibility that these two dikaryons share identical nuclei. The low diversity of genotypes within secondarily infected root systems and the level of polymorphism observed between isolates ARGS2 and BRGS2, suggest that *H. annosum* forms a stable dikaryon during colonization of Christmas trees. Further research is needed to specifically test this hypothesis.

Our data have shown that tree mortality in mortality pockets is caused by many genetically distinct individuals. This is contrasted to the tree-to-tree spread of a single genotype seen in older forest settings (22). Protecting freshly cut stumps from infection by aerially dispersed basidiospores will be a critically important strategy for controlling this disease. This is particularly true in plantations where little or no disease is currently present. Borax has traditionally been applied to freshly cut stumps to limit primary infection in forest situations. This approach works well for pines, but has been inconsistent with spruce (15). Preliminary data from PNW Christmas tree plantations suggest that applications of Sporax (Wilbur Ellis, San Francisco, CA) can protect noble fir stumps from infection if it is applied over the entire surface of the stump immediately after harvest (G. A. Chastagner, unpublished data). Additional research is needed to determine if alternative stump treatments, such as urea sprays, the application of biocontrol agents, or covering stumps with soil can effectively protect them from *H. annosum*. In plantations where *H. annosum* is already causing considerable losses, stumps and root systems need to be removed from the soil or the land will have to be left fallow until residual root systems have decayed and are no longer a potential source of inoculum.

LITERATURE CITED

1. Chastagner, G. A., and Benson, D. M. 2000. The Christmas tree: Traditions, production and diseases. Online. Plant Health Progress. DOI 10.1094/PHP-2000-1013-01-2V.
2. Chastagner, G. A., and Riley, K. L. 2003. Postharvest quality of noble and nordmann fir Christmas trees. HortScience 38:419-421.
3. Chavez, S. J., Jr., Edmonds, R. L., and Driver, C. H. 1980. Young growth western hemlock stand infected by *Heterobasidion annosum* 11 years after pre-commercial thinning. Can. J. For. Res. 10:389-394.
4. Filip, G. M., and Morrison, D. J. 1998. Impact, control and management on *Heterobasidion annosum* root and butt rot. Chapter 23, Pages 405-429 in: *Heterobasidion annosum*: Biology, Ecology, Impact and Control in North America. S. Woodward, J. Stenlid, R. Karjalainen, and A. Hüttermann, eds. CAB International, Oxon, U.K.
5. Garbelotto, M., Fields, W., Cobb, T., and Bruns, T. 1999. Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. Phytopathology 89:546-554.
6. Garbelotto, M., Ratcliff, A., and Bruns, T. 1996. Use of taxon specific competitive priming PCR to study host specificity, hybridization, and intergroup gene flow in intersterility groups of *Heterobasidion annosum*. Phytopathology 86:543-550.
7. Hadfield, J. S., Goheen, D. J., Filip, G. M., Schmidt, C. L., and Harvey, R. D. 1986. Root diseases of Oregon and Washington conifers. USDA Forest Service, Forest Pest Management, R6-FPM-250-86, Portland, OR.
8. Harrington, T. C., Rizzo, D. M., DeScenzo, R. A., and Steimel, J. 1998. Genetic relationships among clones of *Heterobasidion annosum* in a pine forest. Pages 159-170 in: Root and Butt Rots of Forest Trees. C. Delatour, J. J. Guillaumin, B. Lung-Escarment, and B. Marcais, eds. 9th Intern. Conf. on Root and Butt Rots. INRA Editions, France.
9. Johannesson, H., and Stenlid, J. 2004. Nuclear reassortment between

- vegetative mycelium in natural populations of the basidiomycete *Heterobasidion annosum*. Fungal Genet. Biol. 41:563-560.
10. Korhonen, K., Carpretti, P., Karjalainen R., and Stenlid, J. 1998. Distribution of *Heterobasidion annosum* intersterility groups in Europe. Chapter 6, Pages 93-105 in: *Heterobasidion annosum: Biology, Ecology, Impact and Control*. S. Woodward, J. Stenlid, R. Karjalainen, and A. Hüttermann, eds. CAB International, Oxon, U.K.
 11. Kuhlman, E. 1962. A selective media for the isolation of *Fomes annosus*. Phytopathology 52:1310-1312.
 12. Michalakis, Y., and Excoffier, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142:1061-1064.
 13. Morrison, D. J., and Redfern, D. B. 1994. Long-term development of *Heterobasidion annosum* basidiospore infected sitka spruce stumps. Plant Pathol. 43:897-906.
 14. Peakall, R., and Smouse, P. E. 2001. GenAlEx V5: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia.
 15. Pratt, J. E. 1998. Chemical control of *Heterobasidion annosum*. Chapter 14, Pages 260-269 in: *Heterobasidion annosum: Biology, Ecology, Impact and Control*. S. Woodward, J. Stenlid, R. Karjalainen, and A. Hüttermann, eds. CAB International, Oxon, U.K.
 16. Redfern, D. B. 1984. Factors affecting spread of *Heterobasidion annosum* in plantations. Pages 104-114 In: Proceedings of the Sixth IUFRO Conference on Root and Butt Rots of Forest Trees. G. A. Kile, ed. SCIRO, Melbourne, Australia.
 17. Redfern, D. B., Gregory, S. C., and MacAskill, G. A. 1997. Inoculum concentration and the amount of colonization of *Picea sitchensis* stumps by basidiospores of *Heterobasidion annosum*. Scan. J. For. Res. 12:41-49.
 18. Rishbeth, J. 1951. Observations on the biology of *Fomes annosus*, with particular reference to East anglia pine plantations. (III) Natural and experimental infection of pines and some factors affecting severity of disease. Ann. Bot. 15(58):221-246.
 19. Rishbeth, J. 1957. Some further observations regarding *Fomes annosus* Fr. Forestry 30:69-89.
 20. Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.
 21. Smouse, P. E., and Peakall, R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561-573.
 22. Stenlid, J. 1985. Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility and isoenzyme patterns. Can. J. Bot. 63:2268-2273.
 23. Stenlid, J. 1987. Controlling and predicting the spread of *Heterobasidion annosum* from infected stumps and trees of *Picea abies*. Scan. J. For. Res. 2:187-198.
 24. Stenlid, J., and Redfern, D. B. 1998. Spread within the tree and stand. Chapter 8: Biology of *Heterobasidion annosum*. Pages 125-141 in: *Heterobasidion annosum: Biology, Ecology, Impact and Control*. S. Woodward, J. Stenlid, R. Karjalainen, and A. Hüttermann, eds. CAB International, Oxon, U.K.
 25. Sullivan, K. 1998. Incidence of infection and decay caused by *Heterobasidion annosum*. M.Sc. thesis. Oregon State University, Corvallis.
 26. Swedjemark, G., and Stenlid, J. 1993. Population dynamics of the root rot fungus *Heterobasidion annosum* following thinning of *Picea abies*. Oikos 66:247-254.
 27. Witcher, W., and Lane, C. L. 1980. Annosus root rot in slash pine plantations in the sandhill section of South Carolina. Plant Dis. 64:398-399.
 28. Worrall, J. J., Parmeter, J. R., Jr., and Cobb, F. W., Jr. 1983. Host specialization of *Heterobasidion annosum*. Phytopathology 73:304-307.