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Opportunities for Addressing Laminated Root Rot Caused by *Phellinus Sulphurascens* in Washington's Forests

A report to identify approaches and opportunities ripe for research on understanding and managing root diseases of Douglas-fir.

November 2013

Opportunities for Addressing Laminated Root Rot Caused by *Phellinus Sulphurascens* in Washington's Forests

A Report from the
Washington State Academy of Sciences,
In cooperation with the
Washington State Department of Natural Resources

November, 2013

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> To provide expert scientific and engineering analysis to inform public policy making in Washington State, and
>
> To increase the role and visibility of science in the state.

WSAS was formed in response to authorizing legislation signed by Governor Christine Gregoire in 2005. Its 12-member Founding Board of Directors was recommended to the governor by the presidents of Washington State University and the University of Washington, and duly appointed by the governor. In April 2007, WSAS was constituted by the Secretary of State as a private, independent 501(c)(3).

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Opportunities for Addressing Laminated Root Rot Caused by *Phellinus Sulphurascens* in Washington's Forests

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A review and evaluation of a final draft of the report was overseen by the WSAS Report Review Committee, chaired by Anjan Bose. Four independent reviewers–Pierluigi (Enrico) Bonello, Professor, Department of Plant Pathology, The Ohio State University, Columbus, Ohio; Gregory Filip, Regional Forest Pathologist, USDA Forest Service, Portland, Oregon; Everett Hansen, Professor Emeritus, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon; and David Rizzo, Professor, Department of Plant Pathology, University of California, Davis, California–provided very helpful suggestions that the Committee incorporated into the final report.

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Executive Summary

This report from the Washington State Academy of Sciences (WSAS) is in response to a request from the Washington State Department of Natural Resources (DNR) to "identify approaches and opportunities ripe for research on understanding and managing root diseases of Douglas-fir."

Similar to the process used by the National Research Council, the WSAS upon agreeing to undertake a project such as requested by the DNR assembles a committee of state, regional, national, and/or international experts, as needed; develops a statement of task to guide the study; conducts the study; and makes recommendations based on the findings. The Committee that conducted this study included federal (both U.S. and Canadian), state, university, and private company representation from Idaho, Oregon, Washington, and British Columbia.

The statement of task based on the DNR request, followed by further discussions with DNR staff, was:

The Committee will determine which among the full complex of forest tree root diseases is best suited for further scientific research toward applied management options, with a focus on:

- Understanding its economic, environmental, and ecological impacts;
- Past research that has shaped modern understanding of the disease;
- Contemporary management options and recommendations; and
- Opportunities ripe for research, particularly molecular research, with potential to better inform management options.

Root Diseases of Washington Forests

The Committee recognized three major root diseases of Washington forests: *Armillaria* root disease caused by one or more of several biologically distinct species of *Armillaria*; *Heterobasidion* root and butt rot caused by *Heterobasidion annosum* (now *H. occidentale* and *H. irregulare*), and laminated root rot (LRR) caused by *Phellinus sulphurascens* and *P. weirii*.

Armillaria root disease is a worldwide problem and occurs on hardwoods and softwoods alike, although different species occur in different regions and on different hosts. *Heterobasidion* root and butt rot likewise occurs worldwide, mainly on members of the pine family (Pinaceae) but also on some hardwoods. Laminated root rot caused by *P. sulphurascens* occurs in China, Japan, and Russia, but within North America this is uniquely a Pacific

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Northwest disease, occurring in southern British Columbia, Washington, Oregon, northern California, western Montana, and northern Idaho. All hardwoods are immune to both *P. sulphurascens* and *P. weirii*.

All three root diseases are caused by long-term native pathogens that have existed in Washington forests for thousands of years, possibly back to the most recent ice age, thereby making them essentially indigenous, like their tree hosts.

Consensus to Focus on Laminated Root Rot of Douglas-Fir

The Committee chose LRR as the root disease of Washington forests and, more specifically of DNR-managed timber lands that, among the full complex of forest tree root diseases is best suited for further scientific research toward applied management options, for the following reasons.

- LRR caused by *P. sulphurascens* in North America is uniquely a disease of the Pacific Northwest.
- *Phellinus* in Douglas-fir is a relatively simple pathosystem compared with the wider host ranges of *Armillaria* species, for example, with a good possibility for early success to identify instructive comparative information on molecular interactions during the respective parasitic and saprophytic phases of the pathogen's life cycle.
- *Phellinus* clones (genets) are quite stable.
- Committee members know of research sites with high expression of LRR and past monitoring records of treatments and environments (e.g., Aviary, Stand Management Cooperative, Forest Inventory, and Analysis plots).
- *Phellinus* sites can be readily identified using traditional methods (risk mapping, observation, aerial reconnaissance, PCR, record-keeping), and some management practices are available to reduce the impact of this disease.
- The information and conclusions in this report could have international implications because several *Phellinus* species causing decay and mortality of trees (e.g., *P. noxius*, *P. tremulae*) are found in Russia and/or Asia. *Phellinus sulphurascens* also occurs in Russia and Asia (Japan and China) whereas the global distribution of another Pacific Northwest species, *P. weirii*, is not well known.
- The committee considered LRR as a potential model for future studies of the other two root diseases, recognizing that many of the recommendations for management and opportunities for deeper understanding of the *Phellinus*/

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Douglas-fir pathosystem at the molecular, genetic, and genomic levels should serve as models for similar research on *Armillaria* root disease as well as *Heterobasidion* root and butt rot.

Economic, Environmental, and Ecological Impacts of Laminated Root Rot

The primary justification for the DNR to limit losses from root diseases on State-owned forest land is to protect timber production in accordance with the DNR mandate that state timber lands be sustainably managed for income to support schools and other trust beneficiaries, while recognizing the importance of environmental services. The same justifications would apply for privately owned and managed timberland. Because there is interest in, and federal funding for, developing the technology and supply chain to produce jet fuel for commercial and military aircraft in the Pacific Northwest from woody coniferous biomass (www.nararenewables.org/Cached), coniferous biomass in Washington's forests represents another potential source of revenue for the DNR and private landowners. Root disease represents an economic factor that will be a challenge in the development of these potential benefits.

Laminated root rot typically occurs as infection centers that expand radially outward about 30 cm per year. Anticipated climate change could increase the spread rate of the pathogen as well as host susceptibility. Most estimates of the total area of infection centers within a stand consider that the disease is probably 10 m (30 feet) beyond what might otherwise be considered the edge of the infection center as indicated by dead and dying trees. Available research indicates further that losses due to LRR as well as *Armillaria* root disease and *Heterobasidion* root and butt rot in Washington forests are due as much or more to reductions in volumetric tree growth as to outright killing of trees.

The best estimates indicate that economic losses from LRR of coastal Douglas-fir in the Pacific Northwest range from 5% to 15%, which represents a significant impact on DNR revenue. For example, from July 2009 through June 2011, Washington DNR sold approximately 781.5 million board feet (mBF) (1,844,135 m^3) of Douglas-fir from its trust lands in western Washington, with nearly $207 million bid for this timber. Construction of a wood-framed house requires approximately 16,000 board feet (37.8 m^3) of lumber, so harvesting 5% more timber from LRR-affected sites in these two years would have provided an additional 39 million board feet of timber—enough to build 2,437 more houses—and an additional $10,350,000 of revenue to the DNR.

Similar to other disturbance agents, root diseases such as LRR can have an impact on ecosystem services. For example, there is strong evidence that Northwest forests are shifting from being sinks to sources of atmospheric carbon dioxide. Tree mortality and reduced tree growth caused by root diseases may also be altering nutrient cycling, potentially

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leaving nitrogen and water in the soil that could add to nutrient run-off, leaching or loss of nitrogen through conversion to nitrous oxide, another greenhouse gas. Trees killed by root diseases reside as snags for a shorter time than those killed by other agents potentially resulting in reduced availability of nesting sites for snag-dependent birds and animals.

Life History of *Phellinus sulphurascens*

Phellinus sulphurascens on Douglas-fir was originally named *Phellinus weirii*. *Phellinus weirii* was first reported in 1914 on western redcedar in Idaho. The first record on Douglas-fir was in 1929 in British Columbia. It was then recognized that *P. weirii* had two forms (the fir form and the cedar form). Thus, in the older literature, the cause of LRR of Douglas-fir was published exclusively as *P. weirii*. The taxonomy has recently been revised so that the fungus on Douglas-fir and other members of the Pinaceae family is now named *P. sulphurascens*. *Phellinus weirii* is mainly a pathogen on members of Cupressaceae family such as cedars. It is also found on junipers in the Ural Mountains of Russia and western China. Both species occur only on conifers, but there is some evidence of a wider host range, e.g., *P. sulphurascens* has been reported from western redcedar. *Phellinus sulphurascens* and *P. weirii* can be distinguished most reliably by a DNA test (polymerase chain reaction) using species-specific primers. This report focuses on LRR of Douglas-fir caused by *P. sulphurascens*, mainly of coastal Douglas-fir where *P. sulphurascens* has been most important and where most of the information on both the disease and *P. sulphurascens* has been obtained.

Like most root pathogens, *P. sulphurascens* can live both parasitically—by colonizing the live bark and cambium of its hosts—and saprophytically in the wood of dead trees and stumps left after harvest. Once the fungus has penetrated bark tissues, it kills phloem and cambial tissues, and initiates decay in the xylem, advancing progressively into the heartwood and upward in the stem. While there is evidence that the pathogen can continue to advance saprophytically into heartwood after the tree is dead, such advancement into new territory largely ceases upon the tree's death. After infected trees die or are harvested, *P. sulphurascens* progressively retreats from the bark surface of infected roots, leaving behind internal (endotrophic) mycelia that are often protected by zone lines laid down by the fungus. *Phellinus sulphurascens* can survive for decades in stumps left after harvest, but can only grow short distances (millimeters rather than centimeters to meters as done by *Armillaria* rhizomorphs) into soil away from a colonized stump. Instead, the pathogen resides in the stump until a new host root grows to it.

Infection of new trees and spread within stands most commonly involves growth of superficial/surface (ectotrophic) mycelia from intact bark on stumps or old root tissues to new roots of susceptible hosts. Infections may start to become apparent within five to 10

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years after a new stand is established; these continue to progress throughout the life of an infected stand. Vegetative spread of *P. sulphurascens* begins in a stand when roots contact infested stumps or roots left intact in the soil from the previous stand.

Phellinus sulphurascens produces abundant airborne basidiospores on annual, resupinate fruiting bodies. These basidiospores appear to play a role in new infections initiated over the long term—decades if not centuries—as evidenced by the genetic homogeneity within infection centers. The use of restriction fragment length polymorphism (RFLP) analysis to study genetic variation among and between isolates of *P. sulphurascens* (and *P. weirii*) indicates that basidiospore-initiated fusions in established infection centers with potential to alter their genetic profiles are relatively rare events in the short-term epidemiology of LRR. Population structure of *P. sulphurascens*, particularly at the stand level, is among the areas highly recommended for research using the tools of molecular biology. In addition, phylogenetic analysis of both *P. sulphurascens* and *P. weirii* is needed to better understand their evolutionary relatedness and history.

New Approaches to Root Disease Research and Management in the Twenty-First Century

A committee of the National Academy of Sciences, in its report, *A New Biology for the 21st Century*, identified four challenges facing society that have in common the need for greater understanding that will be made possible by the current revolution in the biological sciences. These challenges are: food security; ecosystem function in the face of rapid change; sustainable alternatives to fossil fuels; and individualized or personalized medicine. Maintaining and improving forest health is fundamental to maintaining ecosystem functions in the face of rapid change, and forests are potentially part of the solution to sustainable alternatives to fossil fuels. Healthy forests are also needed to ensure a sustainable supply of timber for the built environment just as healthy crops are critical to food security.

The NAS report points out further that the new biology, while not de-emphasizing more traditional single investigator-initiated research, depends on the involvement of physical scientists and engineers working in an interdisciplinary fashion with biologists. This is consistent with a conclusion in this report that there is an urgent need in the study and control of forest tree diseases generally and root diseases more specifically to attract disciplines outside of the traditional field of forest pathology to get involved in this research. There is an urgent need to reverse the decline in forest pathology in the Pacific Northwest: At present, and due to retirements, no forest pathologist serves on the teaching faculty of either the University of Washington or Washington State University.

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Some of the new emerging and converging areas of science with relevance to forest and root disease management include community ecology, defined as the study of patterns in the diversity, abundance, and composition of species in communities and of the processes underlying these patterns; community genetics, which combines ecology and evolutionary genetics to focus on how intraspecific variation present in foundation (keystone) species impacts functional relationships within particular ecosystems; network theory, which is the analysis of representations of all pairwise interactions among organisms in environments; and ecophylogenetics, which is a fusion of ecology, genomics, and network theory. This later field of study reveals the potential for intraspecific population subdivisions in all network members that play potentially significant roles in the response of forest communities to disturbances.

Even though harvesting of old-growth Douglas-fir began around 1850, managers were generally not aware that an important endemic pathogen was present until 1940, and the extent of the problem was not appreciated until the early 1950s. Modern forest regeneration using seed orchard material dates from the early 1970s. Although growth and yield data indicate that timber productivity can be greatly increased on managed Douglas-fir plantations over wild stands, there are many differences between plantations and natural stands that could influence the host and pathogen community at a molecular or genetic level. Has LRR root rot increased as a result of intensive management of Douglas-fir plantations? There is no documented evidence that harvesting has induced or created new infection centers, but new scientific focus could identify subtle ecological relationships and avert maladaptive conditions before disease expression intensifies and severe disturbances result.

New Biology for the 21st Century investigations have the potential for important benefits ranging from improved stewardship of basic ecological function to intensive timber production. This approach applied to the management of LRR depends on a deeper understanding of the disease that is now within reach thanks to new molecular techniques and computing capacity. Indeed, "biology of the 21st century" is generating an entirely new view of the biosphere that increasingly guides our understanding of the behavior of ecological communities in oceans, waterways, and on land, and can now extended to better understand and address LRR of Douglas-fir in Washington forests and of forest ecosystems more generally.

Recommended Management Strategies for Laminated Root Rot of Douglas-Fir

Fundamental to any management strategy of any forest root disease is to know the location and severity of infection centers on the landscape. The Committee recommends that forest pathologists provide training opportunities, technical assistance, and consultation

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services for DNR foresters who deal with LRR, and that foresters learn to identify LRR, maintain high awareness, and recognize the disease in forest communities.

Remote sensing techniques for pinpointing individual trees with LRR and especially infection centers can include aerial photography, computer-assisted technology, LiDAR, and global positioning systems (GPS), accompanied by ground truthing because several insects, other pathogens, bear damage, and abiotic conditions such as flooding cause trees to produce symptoms similar to those caused by *P. sulphurascens*. Conversely, Douglas-fir beetle mortality around LRR centers can often disguise the presence of root rot. Experienced specialists should be made available when questions occur.

In locating infection centers of LRR within stands, it is important for foresters to record the location of infected trees or stumps prior to making regeneration harvests and to include this requirement in harvest contracts. All managers responsible for the unit should be made aware of the extent of the disease in the stand. This could involve adopting principles of precision agriculture and allowing this data layer to be used in real-time overlays along with other stand and site characteristics.

After conducting a survey, an economic analysis can be done using present net-worth analysis to predict return on investment. Costs of site preparation and stump removal, planting, thinning, vegetation control, and fertilization can be included; and volume per area of land can be predicted with and without LRR. This can take into account the "do-nothing" approach if LRR is not sufficiently damaging, alternative species are not available or economically viable, or funds are not available for treatment.

The DNR could accelerate harvest plans to preempt future mortality by actively cutting around disease pockets, creating non-host tree buffers, and replanting non-host tree species. Any action to starve the pathogen will limit its ability to infect in the future. Thinning very close to infection centers should be done with care because even the remaining trees with green crowns likely will have extensive root rot and be subject to windthrow when the stand is thinned. An investment in traditional thinning practices simply for Douglas-fir density management or growth improvement is unlikely to be recovered.

When reforesting sites known to be infested, tree species suited to the site but less or not susceptible to *P. sulphurascens* and not likely to initiate problems with different pathogens should be considered for replanting. Western white pine, for example, is considered safe for LRR centers, but unless one understands rust resistance and implements practices to reduce potential impacts of this disease, planting this pine will not produce the desired benefit. If no disease treatment is applied, then managers should plan for a shorter rotation and reduce their expected timber yields from the unit. However, a single 30-year rotation of an immune species may not eradicate LRR from a site.

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Information on the occurrence and severity of LRR in a stand is often decoupled from harvest and regeneration decisions and practices under traditional management, although industry typically treats them together. The species resistant to LRR are mostly early seral, with low tolerance of competition. They must seed in and be established before competition from both the abundant and more shade-tolerant susceptible species and herbaceous or shrubby vegetation. To avoid regenerating susceptible species, resistant species must either be regenerated from on-site seed trees, or resistant seedlings must be planted. Seed from resistant sources can be swamped out by vastly more abundant susceptible trees, and the intermittent occurrence of good seed years for resistant species can add to the problem, unless cone crops are monitored prior to harvest. Regeneration through planting seedlings of resistant species takes a minimum of two to three years of planning to ensure that 1) adequate seed with appropriate local adaptation and genetic breadth is available for sowing; and 2) seedlings will be on hand during the critical window for seedlings to establish and compete. Many infected stands get planted back to Douglas-fir because of lack of appropriate resistant regeneration stock. Moving these LRR decisions forward to the pre-harvest phase allows time for nursery staff to plan for production needs. Planting of red alder and western redcedar seedlings is routinely done today with no delay in regeneration.

Stump removal (stumping) is another option to reduce root disease in a regenerating stand by reducing inoculum of *P. sulphurascens* carrying over from one rotation to the next. The major concern with stumping is the balance between costs and benefits of the treatment; it requires very heavy machinery, causes considerable soil disturbance and is limited to slopes (inclines) less than 30%, leaving many forest sites unavailable to this treatment. Stump removal does not eliminate the problem but does lessen mortality in the next rotation. Stump removal may become more attractive because of the increasing trend for Douglas-fir to be grown on shorter rotations, resulting in smaller trees with smaller, more readily removable stumps left after harvest.

Chemical inactivation of *P. sulphurascens* inoculum with fumigants such as chloropicrin has been demonstrated, but fumigants do not penetrate sufficiently into buried roots to adequately eliminate this pathogen. It is not currently used. Moreover, because of cost and restrictive regulatory policies about the use of pesticides in forests, fumigation likely will remain limited to control of tree-seedling pathogens in forest-tree nurseries.

Finally, the Committee recommends that the DNR and private forest managers monitor and share information on treatments for success to determine what works and what does not work. Forest pathologists should assist in establishing and implementing rigorous monitoring protocols and in developing technical assistance guidance information from the conclusions gleaned.

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Recommended Molecular Research on LRR of Douglas-Fir

The Committee agreed that the highest priority for molecular research applied to LRR is to know more about the genetic diversity, population structure, and phylogeography of *Phellinus*. A recent comparative study of *P. sulphurascens* isolates from western North America (Canada and United States) and Eurasia (Siberia and Japan) revealed that *P. sulphurascens* in Eurasia is highly diverse compared with very little diversity among western North American isolates. These results would indicate that Eurasia could be the center of origin of this fungus, and that gene flow appears to be restricted between these two continental regions. However, the western North American isolates, despite representing possibly the largest collection of *Phellinus* isolates in the world, were almost entirely from southern British Columbia. The few Washington isolates studied similarly indicated a single or relatively few clones (genets) of the pathogen, but more research is needed on the question of the pathogen's diversity in North America.

The Committee also recognized enormous opportunities through the use of metagenomics, community genomics/metagenomics and community transcriptomics/metatranscriptomics to not just examine gene expression in the Douglas-fir/*P. sulphurascens* pathosystem but also deduce ecological functions of the critical biotic components of LRR at different stages of the pathogen's life cycle. DNA-based identification of critical microbes within microbial communities in forest soils, and of endophyte communities in host roots at different stages of tree and disease development, would represent a critical first step to understanding biotic interactions that occur within an environment such as the Douglas-fir rhizosphere with potential to explain and eventually exploit any deviations in rate of expansion of infection centers or mortality of trees from what would be expected.

The Committee considered whether some level of natural resistance to LRR might have evolved in Douglas-fir, given that both the host and pathogen are indigenous to the forests of the Pacific Northwest. The conclusion was that even a 10,000-year coexistence would be too short for natural variation and selection pressure to generate and establish resistant phenotypes, considering that each natural rotation of Douglas-fir could take several centuries and selection pressures may be low or intermittent. Susceptible young trees may escape root disease during their first decades of regeneration, so that resistant seedlings are not favored, while older resistant trees may be lost in stand-replacement events, such as widespread Douglas-fir bark beetle attacks or fire.

However, there is evidence from research done in British Columbia of some potentially useful levels of genetic variation in susceptibility/resistance of coastal Douglas-fir to *P. sulphurascens*, and considerable work has been done to characterize the defense response of Douglas-fir to *P. sulphurascens* at the molecular level. Nevertheless, because of the

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long-term nature of conventional tree breeding, and the likely genetic complexity of any resistance so-identified, traditional breeding for Douglas-fir resistance to LRR was not considered high-priority for scientific research toward applied management options to control LRR in Washington forests for the foreseeable future.

On the other hand, the Committee suggests that it may be possible to enhance Douglas-fir resistance through genome modification, e.g., by a customized transcription activator-like effector (TALE) protein. TALEs are DNA-binding proteins, first discovered as the mechanism by which the plant pathogenic *Xanthomonas* bacteria counter the defenses of their host. The DNA-binding specificity of these proteins is determined by two adjacent and highly polymorphic amino acid residues contained within conserved tandem 34-amino acid repeats. The DNA-binding specificity of TALEs is determined by these two amino acid residues, with each two amino-acid residue being specific for a different nucleotide in the targeted genome—one diamino acid residue to one nucleotide. Because of the modular protein architecture of TALEs, it is possible to construct artificial effectors for almost any specificity. This specificity of TALEs has been used to modify genomes of crops, livestock, and embryonic stem cells without the use of recombinant DNA methods. From these successes, TALEs should also be considered for their potential to reduce the susceptibility/enhance the resistance of Douglas-fir to LRR and possibly other root diseases.

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Introduction

Laminated root rot (LRR) caused by *Phellinus sulphurascens* is a disease of Douglas-fir, a "keystone species" for the Pacific Northwest (PNW), both economically and ecologically. This report, produced by a committee (Appendix 1) established by the Washington State Academy of Sciences in response to a request (Appendix 2) from the Washington State Department of Natural Resources (DNR), examines past, present, and future approaches to management of and research on laminated root rot of Douglas-fir in Washington's forests.

This report describes pathogen biology and ecology, economic and environmental impacts of LRR, disease management, and opportunities for research in molecular biology. *Phellinus* in Douglas-fir is a relatively simple pathosystem compared with other root diseases in Washington forests, and it presents the opportunity to reveal new scientific understanding needed to address root diseases of Washington forests more generally. Root diseases have been subject to concerted forest management research since the 1940s. They regulate forest structure and processes, reduce tree growth, kill trees, and otherwise interfere with management objectives. Laminated root rot infection centers can also create centers of diversity in forests, and these are populated with wildlife.

The primary justification for the Washington State DNR to limit losses from root diseases on State-owned forest land is to protect timber production in accordance with the mandate that state timber lands be sustainably managed for income to support schools and other trust beneficiaries, while also recognizing the importance of environmental services. The same justification would apply for privately owned and managed timberland.

This report also recognizes persistent deficiencies in the measurement and understanding of disease impacts and epidemiology, pathogen phylogeny, and host-pathogen interactions. Tools exist to address these deficiencies. Future approaches using molecular and genetic techniques as well as modern theories of community ecology, for example, offer great potential for significant additional benefits in the management realm. Moreover, many of the recommendations for management and opportunities for deeper understanding of the *Phellinus*/Douglas-fir pathosystem at the molecular and genetic levels should be applicable to other root pathogens such as *Armillaria* root disease and *Heterobasidion* root and butt rot.

While written for the DNR, this report should be of interest to federal agencies such as the U.S. Forest Service and National Science Foundation, and to private industry as well as the DNR in funding and implementing the research priorities identified.

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Statement of Task

In correspondence received September 2, 2011 (Appendix 2), the Washington State Department of Natural Resources (DNR) requested help from the Washington State Academy of Sciences (WSAS) to

> evaluate which among the full complex of root diseases [of Douglas-fir] is appropriate for further scientific research toward applied management options . . . [and to focus on] identifying and implementing molecular research on the host-pathogen relationship between Douglas-fir and root diseases such as laminated root rot [caused by] (*Phellinus sulphurascens*) and *Armillaria* root disease.

Consistent with the DNR request, and recognizing that the root diseases in Washington's forests are caused by indigenous pathogens, the Statement of Task agreed upon in consultation with DNR staff is as follows:

The Committee will determine which among the full complex of forest tree root diseases is best suited for further scientific research toward applied management options, with a focus on:

- Past research that has shaped modern understanding of the disease;
- Understanding its economic, environmental, and ecological impacts;
- Contemporary management options and recommendations; and
- Opportunities ripe for research, particularly molecular research, with potential to better inform management options.

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Consensus to Focus the Study and Report on Laminated Root Rot Caused by *Phellinus Sulphurascens*

The Committee recognized three major root diseases of Washington forests: *Armillaria* root disease caused by one or more of several biologically distinct species of *Armillaria*; *Heterobasidion* root and butt rot caused by *Heterobasidion annosum* (now *H. occidentale* and *H. irregulare*); and laminated root rot caused by *Phellinus sulphurascens* and *P. weirii*.

Armillaria root disease is a worldwide problem, and occurs on hardwoods and softwoods alike, although different species occur in different regions and on different hosts. *Heterobasidion* root and butt rot likewise occurs worldwide, mainly on members of the pine family (Pinaceae), but also on some hardwoods. Laminated root rot caused by *P. sulphurascens* occurs in China, Japan, and Russia, but within North America this is uniquely a Pacific Northwest disease, occurring in southern British Columbia, Washington, Oregon, northern California, western Montana, and northern Idaho. *Phellinus sulphurascens* is the major cause of laminated root rot of Douglas-fir and other coniferous species, whereas *P. weirii* principally affects western redcedar and Alaska yellow-cedar. All hardwoods are immune to both *P. sulphurascens* and *P. weirii*. *Phellinus sulphurascens* and *P. weirii* can be distinguished most reliably by a DNA test (polymerase chain reaction) using species-specific primers.

After discussing each of the three important root diseases of Douglas-fir, the Committee, consistent with the Statement of Task to "determine which among the full complex of tree root diseases is best suited for further scientific research toward applied management options," agreed to focus the study and report on LRR caused by *P. sulphurascens*.

This focus on LRR is based on the following:

- LRR caused by *P. sulphurascens* in North America is uniquely a disease of the PNW, occurring mostly west from Montana and from northern California to southern British Columbia.
- *Phellinus* in Douglas-fir is a relatively simple pathosystem compared with the wider host ranges of *Armillaria* species, for example, with a good possibility for early success to identify instructive comparative information on molecular interactions during the respective parasitic and saprophytic phases of the pathogen's life cycle.
- *Phellinus* clones (genets) are quite stable.

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- Committee members know of research sites with high expression of LRR and past monitoring records of treatments and environments (e.g., Aviary, Stand Management Cooperative, Forest Inventory and Analysis plots).
- *Phellinus* sites can be readily identified with traditional methods (risk mapping, observation, aerial reconnaissance, PCR, record-keeping), and management practices are available to reduce the impact of this disease.
- The information and conclusions in this report could have international implications because several *Phellinus* species causing decay and mortality of trees (e.g., *P. noxius*, *P. tremulae*) are found in Russia and/or Asia. *Phellinus sulphurascens* also occurs in Russia and Asia (Japan and China), whereas the global distribution of another Pacific Northwest species, *P. weirii*, is not well known.
- There are no professional forest pathologists currently employed on the teaching faculties of Washington's two research universities. It is critical that this deficiency be addressed. The state is in an excellent position to integrate future forest pathology research efforts with appropriate complementary scientific disciplines and applied forestry activities.
- The committee considered LRR as a potential model for future studies of the other two root diseases, recognizing that many of the recommendations for management and opportunities for deeper understanding of the *Phellinus*/Douglas-fir pathosystem at the molecular, genetic, and genomic levels should serve as models for similar research on *Armillaria* root disease and *Heterobasidion* root and butt rot.

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Estimated Losses from Laminated Root Rot of Douglas-Fir in the Pacific Northwest

Laminated root rot affects the productivity/volume-yield of commercial forests by causing mortality, growth reduction, and butt rot (Figures 1 and 2), and by increasing the susceptibility of infected trees to windthrow and insect damage (Bier & Buckland, 1947; Buckland et al., 1954; Childs, 1963; see also references in Thies & Sturrock, 1995). Loss to root diseases in Washington and neighboring forests may also be a factor along with insect damage in accounting for the evidence that PNW forests are shifting from sinks to sources of carbon dioxide (Kliejunas et al., 2009; Sturrock et al., 2011) at a time when forests are needed for more carbon sequestration not less net. However, damage due to bark beetles, spruce budworm, and fires probably contribute more to the loss of carbon sequestration than root diseases in drier eastside forests.

Figure 1. Crown of a Douglas-fir infected by *Phellinus sulphurascens*.

Note the rounded top, bushy branch ends, and thinning foliage of this tree, which contrast with the healthy firs in the immediate background.

Tree death or reduced tree growth from root disease could be altering nutrient cycling, potentially leaving nitrogen and water unused in the soil that normally would be extracted by tree growth (Hansen & Goheen, 2000). Based on agricultural soil models of crops with root disease (Cook, 1992), this could add to nutrient runoff, leaching, or loss of nitrogen through conversion to nitrous oxide, another greenhouse gas. Root diseases are also involved in the loss of large old trees, recently reported to be occurring on a global scale with major implications for ecosystem integrity and biodiversity (Lindemayer et al., 2012). And trees in western Washington and Oregon killed by root diseases reside for a shorter time as snags than trees

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killed from other reasons, which can reduce availability of nesting sites for snag-dependent birds and animals (Ohmann, 2002).

The impact of LRR on the productivity of managed forest stands has been estimated by several investigators (e.g., Buckland et al., 1954; Bloomberg & Wallis, 1979; Tkacz & Hansen, 1982; Lawson et al., 1983; Thies, 1983; etc.), all of whom emphasize the importance of identifying and managing the disease(s) as key to accurate forecasts of potential and, hopefully, realized yields. Even an "ecosystem management" approach may not be possible in many of today's forests due to the effect of human activities and interventions on LRR dynamics. For example, planting monocultures of susceptible species and excluding fire in eastern Washington, where natural fire return intervals are short, can generate or leave intact greater amounts of *P. sulphurascens* inoculum in managed forested stands than normally would be found if stands had been "self-managed" (Wallis & Reynolds, 1965; Thies & Sturrock, 1995; Hansen & Goheen, 2000). In western Washington, windthrow in natural stands may have led to less LRR because root systems were uprooted, and fire exclusion is less important because of the long fire return interval. In addition, several researchers (van der Kamp, 1991; Hansen & Goheen, 2000; Durrall et al., 2005; Winder & Shamoun, 2006), while presenting an ecological perspective, have reminded us that forest pathogens play key roles in determining forest structure and processes in both wild and managed forests of western North America, and so may be beneficial in some settings while being detrimental in others.

Figure 2. Canopy gap, and dead and dying Douglas-firs, characteristic of disease centers associated with laminated root rot caused by *Phellinus sulphurascens*.

Phellinus sulphurascens is considered to be a significant predisposing agent, weakening trees and making them more attractive to attack, and enabling successful colonization by Douglas-fir beetles (*Dendroctonus pseudotsugae* Hopkins) (Childs, 1963), fir engravers (*Scolytus* spp.) and other insects (Hadfield et al., 1986; Goheen & Hansen, 1993; Thies & Sturrock, 1995). Trees infected by *P. sulphurascens* also serve as food to help maintain endemic beetle populations between outbreaks. While fires do not directly affect the

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survival of *P. sulphurascens* inoculum in infection centers, sites in drier environments with high fire frequency have a recurrent alternation between shade-intolerant "early seral" species that are immune or of low-susceptibility (e.g., hardwoods, pines) and later, more shade-tolerant and LRR-susceptible species (true firs and mountain hemlock), including Douglas-fir (McCauley & Cook, 1980). Damage from LRR is expected to be low in forests with recurrent fire, but where fire is excluded by human interventions, susceptible coniferous species and hence LRR may predominate (Thies & Sturrock, 1995). In western Washington where fire is rare (Agee, 1998), Douglas-fir sites with LRR may have immune early-successional hardwood species such as red alder or vine maple or less susceptible late-successional conifers such as western redcedar or western hemlock. Laminated root rot may persist for long periods in Douglas-fir stands on edaphic climax sites for Douglas-fir or in the roots of less susceptible hosts.

Range and Intensity of Infection Centers

Phellinus sulphurascens occurs throughout the range of Douglas-fir in Oregon, Washington, Idaho, Montana, California, and British Columbia. Estimates as to the area of land it occupies (as infection centers; Figure 2) vary, but it appears to be less than 15% across the region as a whole (Table 1).

Table 1. Laminated root rot infection percentages in Douglas-fir stands in Washington and Oregon.

Location	Area with LRR (%)	Reference
Western WA and OR	5	Lawson et al. (1983); Hadfield (1985)
Western WA and OR	8-11	D. J. Goheen (unpublished)
Northwest OR Non-Federal Timberlands	13-14	Gedney (1981)
Coast Range OR	5.6 (range 0-14)	Kastner et al. (1994)

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Reports of LRR in Douglas-fir stands of western Washington and Oregon range from about 5% (Lawson et al., 1983; Hadfield, 1985) to nearly 11% (D. J. Goheen unpublished data). Goheen (personal communication, D.J. Goheen, U.S. Forest Service) estimated that LRR occurs on 8% of the commercial forest land in Washington and Oregon. Kastner et al. (1994) found the overall infection level averaged 5.6% in 70- to 100-year-old stands of Douglas-fir in the Nestucca River drainage of coastal Oregon, but it varied from 0 to 14.7%. Gedney (1981) estimated the occurrence of LRR on non-Federal timberlands in northwest Oregon to be between 12% and 13%. However, only dead trees were used to indicate the presence of the disease in this survey, so the actual occurrence of the pathogen likely was much higher.

Bloomberg & Beale (1985) found that soil moisture was important with respect to the occurrence of infection centers. The number and length of centers per 100 m of site unit varied greatly but was lowest in very wet or very dry soils and highest in dry or moist to slightly dry soils. Kastner et al. (1994) found similar results. However, Bloomberg (1990) reported that LRR centers show consistent radial expansion of the disease across a broad ecological range estimated to be ~35 cm/year. Variation in rate of radial spread on a stand basis can be explained by the planting density of Douglas-fir, density of resistant or tolerant species, and inoculum density of the pathogen. Bark beetles are typically associated with *P. sulphurascens*. Lane & Goheen (1979) found that the incidence of *P. weirii* was also correlated with bark-beetle losses in true firs. In the Colville and Wenatchee National Forests, *P. sulphurascens* was found in 7% and 44% of bark beetle mortality centers, respectively.

Estimated Losses

A number of estimates of losses due to *P. sulphurascens* in Oregon, Washington, and British Columbia were published between 1949 and 1983 (Table 2), but no good estimates have been published since that time, including for managed stands.

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Table 2. Estimates of annual timber losses and percentage of annual productivity in Washington, Oregon, and British Columbia due to *Phellinus sulphurascens*.

Organism	Location	Annual loss ft^3 x 106 m^3 x 10^6		%	Reference
All root rots	Western WA & OR	83	2.35	2.67	Childs & Shea, 1967
P. sulphurascens	Eastern WA & OR	32	0.91	1.03	Childs & Shea, 1967
	115		3.70		
P. sulphurascens 1977	WA & OR	32	0.91		Hadfield & Johnson,
P. sulphurascens 1977	BC	37	1.05		Hadfield & Johnson,
P. sulphurascens	BC	35	1.00		Wallis, 1967
P. sulphurascens	Northwest US & BC	157	4.44		Nelson et al., 1981
P. sulphurascens	WA & OR			5.00	Childs, 1949
P. sulphurascens	40-year-old OR			13a	Thies, 1983
				7b	Thies, 1983
				32c	Thies, 1983
				12d	Thies, 1983

a - growth loss in last 10 years - living infected trees
b - volume loss in last 10 years - living infected trees
c - growth loss in last 10 years - dead LRR-killed trees
d - volume loss in last 10 years - dead LRR-killed trees

The percentage loss of annual productivity was estimated to be < 5%, except in heavily infected stands, where it is much higher (Thies, 1983). Losses caused by various groups of diseases in PNW forests were estimated in 1954 (U.S. Forest Service, 1958). In the 1960s, a more detailed analysis was performed by Childs & Shea (1967), who suggested that all diseases reduced the annual productivity of Oregon and Washington's forests by 13%. The annual growth loss of 403 million ft^3 (11.4 million m^3) consisted of 162, 129, and 112 million ft^3 (4.6, 3.6, and 3.7 million m^3) of lost growth, mortality, and cull, respectively; the cull was mostly in old-growth forests. Root diseases contributed annual losses of 115 million ft^3 (3.3 million m^3) in mortality and growth loss (or 3.7% of annual productivity) (Table 2). Of the 115 million ft^3 (3.3 million m^3) annual growth loss, about 32 million ft^3 (0.9 million m^3) occur in west-side Douglas-fir alone; overall, west-side losses from root rots were estimated at 83 million ft^3 (2.4 million m^3) compared with east-side losses of 32 million ft^3 (0.9 million m^3).

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Goheen & Hansen (1993) estimated that *P. sulphurascens* can cause a 40% to 70% reduction in wood volume in the affected areas. Using regression analysis of 40-year-old Douglas-fir, Thies (1983) found a 13% growth loss and 7% volume loss for living infected trees in the 10 years prior to harvest, compared with 32% and 12%, respectively, for trees killed by the disease. Thies (1983) also estimated a mean growth loss of infected trees of 2% of predicted harvest volume and 8% of the last 10 years' volume growth.

Wallis (1959) suggested that the greatest losses to *P. sulphurascens* were in stands 30-150 years old. Total annual losses caused by *P. sulphurascens* in Washington and Oregon are reported to be 32 million ft^3, with another 37 million ft^3 (0.9 million m^3) being lost in British Columbia (Hadfield & Johnson, 1977). Losses caused by tree mortality and lost growth have been estimated at 1.4 million m^3 (49.4 million ft^3) (Sturrock & Garbutt, 1994). Childs & Shea (1967) estimated losses of 0.9 million m^3 (31.8 million ft^3) in Washington and Oregon, while Wallis (1967) estimated losses of 1 million m3 (35.3 million ft^3) in British Columbia as cited by Thies (1983). Nelson et al. (1981), Thies (1982), and Thies & Nelson (1982) estimated annual losses at 4.4 million m^3 (157 million ft^3) of timber in northwestern USA and British Columbia.

Second-growth stands began to be established soon after old-growth Douglas-fir forests were first cut in the 1850s. Childs (1949) noted that, overall, *P. sulphurascens* could cause 5% loss of productivity in second-growth stands in western Oregon and Washington. Substantial reductions in timber volume and growth have been demonstrated in many second-growth Douglas-fir stands from Oregon to British Columbia (Mounce et al., 1940; Buckland et al., 1954; Johnson et al., 1972; Bloomberg & Wallis, 1979; Nelson, 1980; Bloomberg, 1981; Bloomberg & Reynolds, 1985; Thies, 1982; Lawson et al., 1983; Thies, 1983). Losses in timber volume due to *P. sulphurascens* are most conspicuous as mortality or windthrow, but tree growth also may be reduced for many years before death (Gillette, 1975; Thies, 1983; Bloomberg & Reynolds, 1985).

More than 50 years ago, Buckland (1955) wrote on predicting losses from root diseases. The excerpt below shows how little our abilities have changed.

> There is a great tendency among foresters to distrust estimates of current damage or predictions of future damage by entomologists and pathologists. This distrust stems largely from our inability to come up with results which are sufficiently representative for wide application and from our inability to present findings in terms and units of measure which are amenable to practical application by the forester: Frequently the samples have been too small or too local to form a basis for a good estimate, or predictions have been based on too pessimistic understanding of the ability of nature to recuperate. In

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> many cases too, the estimates have been based on board-foot loss per acre alone, the most easily computed figure, without regard for the distribution of the remaining stems, the influence of the disease on crown class distribution, or some other factor frequently difficult to measure.

In the Pacific Northwest, we are still far from being able to appraise or predict damage caused by root diseases. Measuring root disease incidence and severity is a challenge, as illustrated by the lack of consistency in the numbers presented here. A seemingly trivial yet very significant problem is how to set the boundaries of individual infection centers. Do you measure to the furthest dead tree, the edge of the crown of the first live tree, or the bole of the first live tree, or do you add one or two more trees to capture the still hidden root infections? A clear recommendation is important for future measurements. With management decisions properly based on cost-benefit analysis, it is essential that meaningful loss figures be available at the stand as well as regional scales. This should be identified as a high priority need for future management.

Offset of Growth Losses Due to *P. sulphurascens*

Because *P. sulphurascens* causes mortality and stand thinning, some of the growth losses due to *P. sulphurascens* may be offset by increased growth of the remaining uninfected Douglas-firs and less susceptible species in the stand. However, trees on the edge of mortality centers are likely to be infected and suffering growth losses. The largest trees on the edges are statistically most likely to die, because their larger root systems have a higher chance of contacting inoculum. This makes offset of growth losses hard to estimate and was first considered by Buckland (1955) in the following excerpt from the WIFDWC proceedings.

> One of the very knotty problems in damage appraisal is encountered in studies of root rot caused by *Poria weirii*. It has been the practice to work out losses on a per-acre basis and the results of such estimations appeared in one annual report containing summations of disease and growth and yield of Douglas-fir. It was interesting to note that the annual loss expected for *Poria weirii* was exactly equal to the annual loss expected for maintaining normal stocking. A forester might come to the conclusion from these results that the root rot was a beneficial rather than a destructive agency as it appeared to thin the stand to maintain normal stocking. The difficult factor to measure is the influence of stocking and its long term effect. The Cowichan Lake Experimental Forest is riddled with *Poria weirii*, yet foresters have mentioned that they did not feel the disease had an appreciable effect on the final yield in the area. Twenty years ago, the centers of infection looked very bad but

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> now they do not look particularly serious as the crown canopy has almost closed and the uneven distribution of stems is not so apparent. The disease is having a serious effect on the stand, however, as many of the trees marked for final crop have gone out, leaving trees of lower dominance and leaving small openings in the 55-year-old stand. Thus, estimates of damage must take into account the loss of dominant trees and the uneven distribution of stems rather than base findings on simple annual loss per acre figures.

Forty years later, Thies & Sturrock (1995) concluded that:

> Reduced volume of preferred timber species due to disease mortality or growth loss may be partially offset by regeneration of less susceptible species in the created openings or by the increased growth of adjacent susceptible, but uninfected, residual trees (Oren et al., 1985). Regardless of its effects, laminated root rot is a significant natural force to be considered by resource managers, whether planning for individual stands or entire ecosystems. Society today demands an increasingly broad range of forest products and experiences, including an ever-increasing volume of fiber from a continually shrinking base of commercial forest land. Understanding and properly managing laminated root rot is imperative to achieving desired management objectives.

Landscape Detection and Assessment

Effective management of root rots of Douglas-fir requires detection and quantifying impacts on a landscape scale. A 1993 root-disease survey of 2,500 acres conducted in southwest Washington by Browning et al. (2002) found that 67% of tree mortality detected by aerial survey was attributed to *Phellinus* or *Armillaria*. From a random subsample of 35 root-disease centers representing three age classes from 22 to 67 years, stand-age class was unrelated to the level of observed mortality (Table 3). Root-disease centers containing more than 10 dead trees per center represented only 14.3% of the centers detected, while 42.9% were small (1-3 trees per mortality center) (Table 4). The study concluded that infection centers might have a high incidence but low severity in younger managed stands (20-60 years). However, when detected, stands in this age-class with greater than 10% mortality from root rot should be diverted from normative silvicultural prescriptions and considered for early rotation harvest, species shift, or other management goals (e.g., wildlife habitat).

It has generally been assumed that there are two patterns of LRR distribution in a stand; either scattered small patches or a few larger ones. But is the "mortality center model" of distribution, with areas increasing radially through root-to-root contact, accurate? This

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is based on assumptions of clonal spread and rare spore infections. Although consistent with many observations, the "mortality center model" has seldom been documented. In some stands, infections seem to be scattered, without obvious foci. Could this be an early stage in disease development or a reflection of multiple spore infections? Dickman & Cook (1989) suggested that the establishment of small young *P. sulphurascens* genets in mountain hemlock stands in the Oregon Cascades were from basidiospores. Basidiospore establishment is an important question to answer and essential for disease management. Studies of population structure at the stand level should have high priority.

In short, it appears that we do not have very good estimates of infection levels and growth losses due to *P. sulphurascens* in Washington. Those that have been published are rough and old, but are still repeated in publications. Infection levels are estimated to be < 15%, and annual productivity losses in second-growth stands are less than 5%, except in heavily infected stands.

Table 3. Average impact and number of trees killed per infection center in 35 randomly detected root rot in various stand age classes in southwest Washington.

Stand age (yrs)	No. centers	Average impact area (acres)	No. trees killed per center
22-28	7	0.09	13
33-40	16	0.04	4
53-67	12	0.07	4

Table 4. Number and percentage of root rot centers containing 1-3, 4-6, 7-10, and >10 dead trees per center.

No. of dead trees per center	No. of centers	Percentage of centers
1-3	15	42.9
4-6	9	25.7
7-10	6	17.1
>10	5	14.3
Total	35	100.0

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Economic Cost of Laminated Root Rot of Douglas-Fir in Western Washington

Obtaining an estimate of the cost of root diseases, whether in agriculture or forestry, is difficult because of their subtle but highly significant effects on growth and development of the infected plants. Except in cases of outright killing, trees (or crop plants) with root disease are typically smaller in stature, produce fewer branches, and may appear nutrient deficient, but otherwise tend to look no different than healthy plants in the same stand and at the same age.

One advantage when estimating losses caused by LRR is that diseased trees commonly occur as patches known as "infection centers." These centers can be located by the dead or obviously dying trees in the center of the patch surrounded by trees of varying but generally smaller stature than those clearly outside the patch. Some estimates have placed the reduction in wood volume within infection centers as great as 40% to 70% (Goheen & Hansen, 1993). Overall losses within a stand are then estimated based on the area within the infection centers relative to the total area of the stand. These estimates done by different investigators and mainly for Douglas-fir west of the Cascades over a 40-year period have ranged consistently between 5% and 15% (Childs, 1963; Bier & Buckland, 1947; Buckland et al., 1954; Thies, 1983; see also references in Thies & Sturrock, 1995).

While any negative effect of a pest or disease in agriculture and forestry can be referred to in the scientific literature as a "loss" whether it occurs pre- or post-harvest, effects that cause pre-harvest decreases in survival and volumetric growth from the continuum expected for that site and stand age are more correctly considered "shortfalls" in expected harvest. Thus, for every 1 million board feet (mBF) (2,360 m^3) of saw logs actually harvested, for example, the yield without the 5% shortfall should have been 1,050,000 mBF (1mBF x 1.05) (2,478 m^3), and that without an estimated 15% shortfall should have been 1,150,000 mBF (1 mBF x 1.15) (2,714 m^3).

The DNR sold 781.5 mBF (1,844,135 m^3) of Douglas-fir from Trust lands in western Washington for a bid of nearly $207 million during the 24-month period from July 2009 to June 2011 (raw data provided by DNR). Assuming a 5% shortfall, the harvested yield should have been 820.575 mBF (1,936,341 m^3) and brought in $217.3 million at the same bid. Similarly, with a 15% shortfall, the harvested yield should have been 898.725 mBF (2,120,755 m^3) and brought in $238 million in revenue.

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While an overall shortfall of 15% would apply to specific sites and not the entire area harvested by the DNR during that 24-month period, LRR is particularly destructive on Douglas-fir in western Washington, and an overall estimated 5% shortfall due mainly if not entirely to *P. sulphurascens* is well within the level of damage caused by this pathogen on coastal Douglas-fir.

It requires about 16,000 BF (37.8 m^3) to build a three-bedroom house. On this basis, the additional harvested yield of 39 mBF (92,030 m^3) assuming a 5% shortfall would have produced enough additional lumber to build 2,442 more houses. According to school projects designed by TCF Architecture of Tacoma, Washington, the wood required for framing and finishing a wood-framed school amounts to about 20% of the hard cost of construction, with today's construction cost for a school in the range of 60,000 SF costing $15 to $20 million. Assuming that $7.5 million of a $10 million shortfall because root disease is added to the Trust for schools after management fees and overhead are subtracted, this additional revenue would pay for the wood for three to four more schools.

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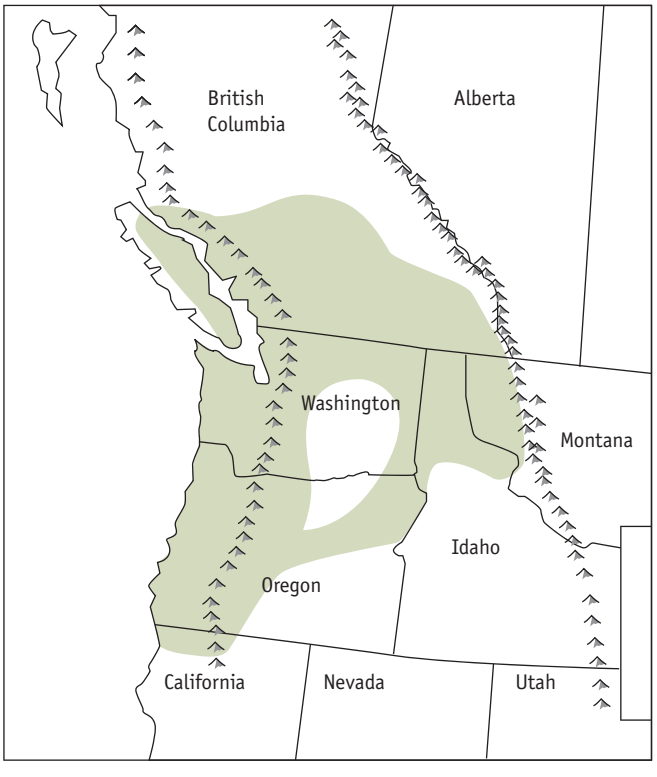
Overview of Laminated Root Rot of Douglas-Fir and Life History of *Phellinus Sulphurascens*

The Causal Organism

Laminated root rot (LRR) of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco; DF], caused by the fungus *Phellinus sulphurascens* Pilát [syn. *P. weirii* (Murrill) Gilb. - Douglas-fir form (Larsen & Cobb-Poulle, 1990; Lim et al., 2005)], occurs in southern British Columbia (BC), Washington, Oregon, northern California, western Montana, and northern Idaho (Figure 3) (Buckland et al., 1954; Larsen et al., 1994; Thies & Sturrock, 1995). *Phellinus sulphurascens* is also found in Japan (Aoshima, 1953), China (Dai & Qin, 1998; Dai, 2010), and Siberia (Parmasto & Parmasto, 1979; Kotiranta et al., 2005). A recent report of its collection from juniper stumps (*Juniperus excelsea* and *J. foetidissima*) in Turkey (Doğan & Karadelev, 2009) needs confirmation.

Figure 3. Combined and overlapping distributions of *Phellinus sulphurascens* and *P. weirii* in western North America.

Phellinus sulphurascens causes laminated root rot of mainly Douglas-fir and other conifers; *P. weirii* causes cedar laminated root and butt rot of western redcedar and yellow-cedar (Thies & Sturrock, 1995). Douglas-fir and western redcedar are sympatric across much of the geographic area denoted here, so host alone should not be used for definitive identification of either *Phellinus* species.



In North America, a closely related species, *P. weirii*, occurs on cedars (Murrill, 1914), including in southeastern Alaska (Hennon, 1991; Sturrock et al., 2010), southern interior and coastal British Columbia, Washington, Oregon, and northern and central Idaho (Thies & Sturrock, 1995) (Figure 4). *Phellinus weirii* is also found on species of juniper in the Ural Mountains of

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Russia and in western China (Dai, 2004). *Phellinus weirii* principally affects species of the Cupressaceae family [e.g., western redcedar (*Thuja plicata*) and yellow-cedar (*Callitropsis nootkatensis*)] whereas *P. sulphurascens* affects several genera of the Pinaceae family (e.g., *Pseudotsuga, Abies, and Tsuga* spp.). All hardwoods are immune to both *P. sulphurascens* and *P. weirii* (Thies & Sturrock, 1995). *Phellinus weirii* and *P. sulphurascens* can be distinguished most reliably by polymerase chain reaction (PCR) using species-specific primers (Lim et al., 2005) and are commonly referred to, respectively, as the redcedar and non-cedar form of the LRR pathogen. A comprehensive phylogenetic analysis of *P. weirii* and *P. sulphurascens* using many isolates from different hosts and locations in Washington, Oregon, and British Columbia is well overdue. It is also important to clarify the host ranges of *P. weirii* and *P. sulphurascens*. It may not be a simple cedar vs. Douglas-fir difference. Western redcedar growing in the midst of LRR of Douglas-fir may well be infected by *P. sulphurascens*.

Phellinus weirii sensu lato was originally described in 1914 on western redcedar in Idaho (Murrill, 1914). The first record of *P. sulphurascens* on Douglas-fir dates back to 1929 from specimens collected in the Cowichan Lake area of BC (Mounce et al., 1940). Because this report is focused on LRR of Douglas-fir, it also consistently refers to the pathogen as *P. sulphurascens*. This overview focuses further on LRR of coastal Douglas-fir where *P. sulphurascens* has been most important and where most of the information on both the disease and *P. sulphurascens* has been obtained.

The geographic distribution of *P. sulphurascens* matches well with the northern, western, and eastern ranges of coastal and interior Douglas-fir. While interior Douglas-fir occurs southward into north and central Mexico (Burns & Honkala, 1990), *P. sulphurascens* has only been found as far southeast as a point approximately midway along the Idaho-Montana border (Figure 4). The farthest southern extension of *P. sulphurascens* occurs in coastal Douglas-fir in northern California near the Oregon border. Additional survey work is needed to confirm the range distribution of *P. sulphurascens*. Plots could be established to monitor for shifts in this distribution under changing environmental/climatic conditions.

Research on root diseases has lagged behind that for above-ground diseases for several reasons. Other than when the host has been killed, symptoms associated with reduced growth caused by a diseased root system are easily attributed to other factors, such as lack of soil fertility, other soil problem, or an abiotic stress. Research on root diseases is also inherently more difficult than for above-ground diseases because of the need to excavate and separate roots from soil, and the complexity of the microbiological environment of soil, the rhizosphere, and roots compared with foliage. These challenges are compounded when working on trees compared with agricultural crops. The elusive root pathogens responsible for the apple "replant disease," for example, have only recently been identified

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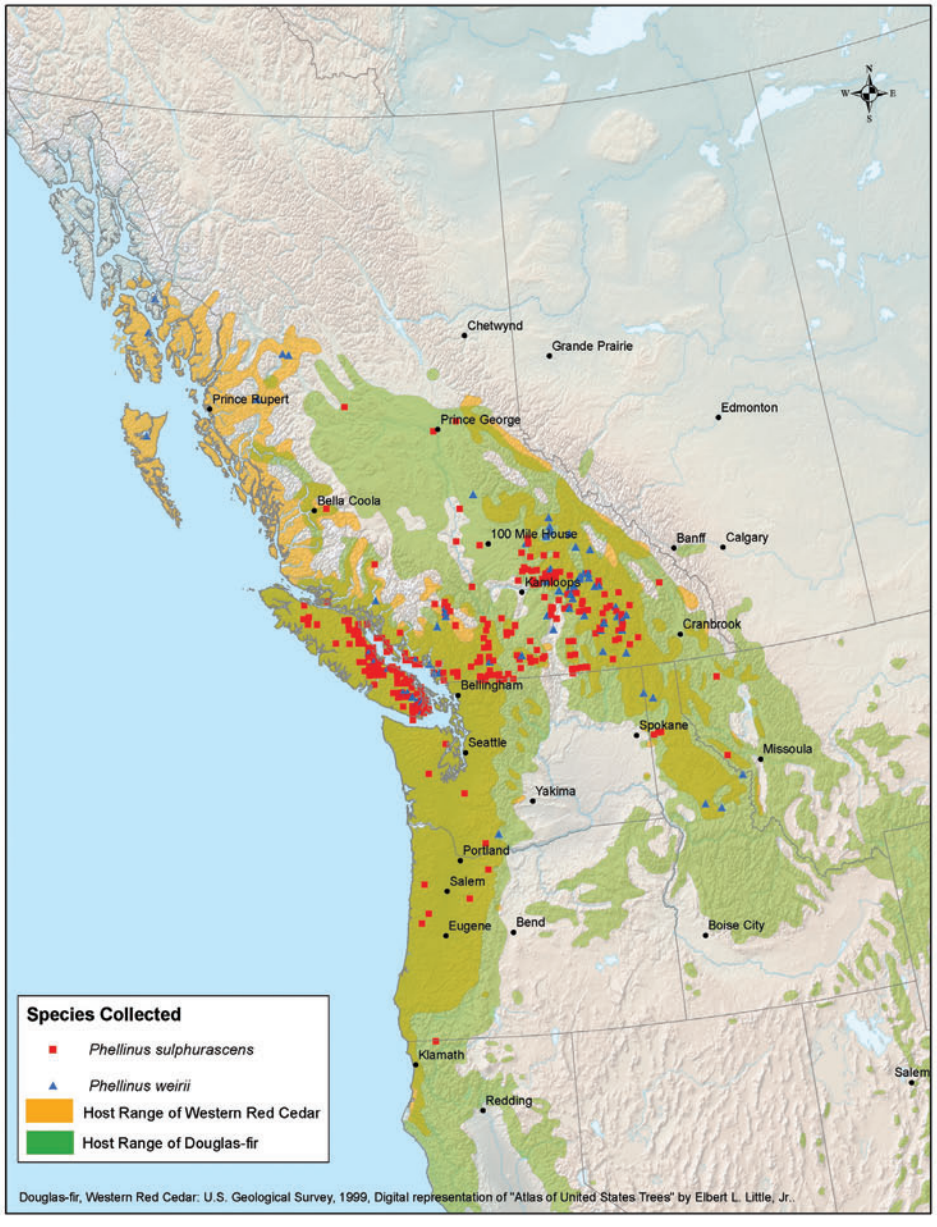
Figure 4. Map of western North America showing almost 600 specific locations where *P. sulphurascens* and *P. weirii* have been confirmed occurring on several different coniferous hosts.

Canadian data were collected mostly by Canadian Forest Service staff and are focused on locations in BC. Location data and isolates from the USA were contributed by pathologists from the USDA Forest Service. The Sturrock lab has a collection of live isolates representing 124 of the *P. sulphurascens* locations, about half of which have been confirmed molecularly, and 48 of the *P. weirii* locations, about half of which have been confirmed molecularly.



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after decades without a diagnosis of this disease complex (Mazzola & Manici, 2012). Also, two or three different root disease-causing pathogens are often present in forests prone to losses from root disease, even on single root systems.

Nevertheless, much is known about the life cycle of *P. sulphurascens*. As a facultative parasite, *P. sulphurascens* has both a parasitic stage in its life cycle, i.e., it can live in and obtain its nourishment from living tissue, and a saprophytic stage, i.e., it can live in and obtain its nourishment from dead tissue. Indeed, the case can be made that it uses its ability as a parasite to be the primary colonist of root and wood tissue that will eventually become dead roots and stump and thereby, as first in, preempt colonization by the strict saprophytes, i.e., those that can only live on dead tissues and must wait for the tree to die before they can begin to colonize the wood.

It is thought that *P. sulphurascens* and most other facultative parasites cannot use their ability as saprophytes to colonize any part of the dead plant not colonized through parasitism while those plant parts were still alive, i.e., the "territory" occupied through parasitism must suffice as the limits of the organism's food base until it can infect another tree. While there is evidence that *P. sulphurascens* is able to colonize sapwood and heartwood after the tree dies and expand its territory, such advancement largely ceases with death of the tree and because of encounters with other microorganisms now able to colonize these tissues. These "other" microorganisms represent a range of abilities from weak parasites to strict saprophytes, including endophytes, which may have already moved into those parts of the plant not colonized by the pathogen earlier through parasitism while the tree was still alive. Indeed, a pathogen's ability to survive as a saprophyte means it now has to maintain possession of a food base (Bruehl, 1975). *Phellinus sulphurascens* is apparently able to maintain possession of its food base during it saprophytic survival by sealing out other wood invading fungi in stump and bole tissue.

Knowledge that *Heterobasidion annosum* (in the broad sense; formerly, *Fomes annosus* and now called *H. irregulare* and *H. occidentale* in North America) is fully capable of colonizing stumps of healthy pine trees if not preempted by other organisms led to a novel biological control of annosus root and butt rot on pine in the United Kingdom (Rishbeth, 1963). When it was discovered that the pathogen colonized freshly cut stumps by airborne basidiospores released from its reproductive structures on root-rotted trees, and used the colonized stumps to infect neighboring trees by colonizing down the roots and across root grafts to neighboring live trees, Rishbeth (1963) showed that inoculating stumps with *Peniophora [Phlebiopsis] gigantea* (a saprophyte/weak parasite) immediately after tree-felling prevented *H. annosum* from colonizing the freshly cut stump. Various formulations and methods of applications of this biological control are currently applied on an estimated 47,000 ha annually in Nordic countries (Thor, 2003). Apart from the axiom that "possession is nine-tenths of the law" (Bruehl, 1975), recent evidence indicates that *P. gigantea*

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induces a localized defense response in the still-living wood; this is shown by an increase in phenolics and lignified cells that served to limit subsequent colonization of the stump by *H. annosum* as well as *P. gigantea*.

Unfortunately, this method of biological control will not work for LRR because *P. sulphurascens* infects healthy trees through roots as they grow near a colonized stump or root. Also, while *P. sulphurascens* produces basidiospores from fruiting bodies, these spores are thought to rarely establish new infection centers.

Morphological and Biological Characteristics of *P. sulphurascens*

Microscopic and culture characteristics of *P. sulphurascens* are summarized in Table 5; more detailed information can be found in several publications (e.g., Mounce et al., 1940; Larsen & Cobb-Poulle, 1990; Larsen et al., 1994).

Table 5. Summary of characteristics for *Phellinus sulphurascens* and laminated root rot occurring in North America.

Characteristics	Description
Hosts	Usually on conifer hosts other than western redcedar (e.g., Douglas-fir, grand fir, mountain hemlock, western hemlock, pacific silver fir, white fir)
Pathogenesis	Infection and decay of roots of susceptible host species frequently results in whole tree mortality; host dies standing or is windthrown before death; characteristic "rootball" associated with windthrow
Decay - incipient/early	Reddish-brown to chocolate brown irregular patches or crescent-shaped stains on fresh stump tops or cross sections of large roots
Decay – advanced	Often evident on stumps tops or ends of broken roots on windthrown trees; laminated decay with oval pits (0.5 x 1 mm) that easily separates along annual rings; layers or clumps of setal hyphae often evident between layers of decayed wood
Fruiting bodies/ Basidiocarps	Resupinate (flat), pore-covered crusts that form on undersides of fallen trees and uprooted stumps; initially light gray-brown aging to uniform chocolate brown; pores small and somewhat irregular in outline; usually annual, though are inconspicuous/dif-

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	ficult to find and/or are rare
Hyphal system	Monomitic; generative hyphae simple septate
Mycelium – homokaryotic	Multinucleate (mean of 3.2 nuclei per cell)
Mycelium – heterokaryotic	Irregularly binucleate (mean of 2.2 nuclei per cell)
Basidiospores	Uninucleate, with one germ tube; hyaline, ovoid, smooth, 4.5-6 x 3.5-4.5 µm
Sexual compatibility/ mating system	Heterothallic; bipolar (governed by single gene locus) and multiallelic; does not form clamp connections
Sexual incompatibility	Single-spore (SS) isolates from different basidiocarps sexually compatible; most SS from same basidiocarp not compatible
Somatic incompatibility	Regulated at one or two multiallelic loci; manifested in culture by a line of demarcation
Population structure	Clonal with old, individual genets spreading vegetatively
Synonyms	Authority
FomitoPoria weirii	Murrill, 1914
Poria weirii	(Murrill) Murrill, 1914
Phellinus sulphurascens	Pilát, 1936
FuscoPoria weirii	(Murrill) Aoshima, 1953
Inonotus weirii	(Murrill) Kotlaba & Pouzar, 1970
Phellinus weirii	(Murrill) Gilbertson, 1974
Inonotus sulphurascens	(Pilát), Larsen, Lombard, & Clark, 1994
Phellinidium sulphurascens	(Pilát), Dai, 1995

Phellinus sulphurascens is a heterothallic basidiomycete and, like all members of the Hymenochaetaceae, lacks clamp connections (Gillette, 1975; Hansen, 1979a). Single-spore isolates (homokaryons) of *P. sulphurascens* are multinucleate, whereas isolates from sporophore tissue (heterokaryons) are irregularly binucleate (Hansen, 1979a). Both homokaryons and heterokaryons can infect wood and cause decay, although the majority of isolates recovered from decayed roots and stumps are heterokaryons (Hansen, 1979b; Lim et al., 2008).

The genetic system governing mating compatibility in *P. sulphurascens* is bipolar (governed by a single gene locus) and multiallelic (Angwin & Hansen, 1993). The use of

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restriction fragment length polymorphism (RFLP) analysis (Bae et al., 1994) to study genetic variation among and within isolates of *P. sulphurascens* (and *P. weirii*) indicated that initiation of infection centers by spores and subsequent vegetative initiated fusions in established infection centers that would alter their genetic profiles are relatively rare events in the epidemiology of LRR.

Phellinus sulphurascens produces its basidiospores from an annual, resupinate fruiting body (Figure 5). Spores appear to play a role in initiating new infections more commonly over the long term (Nelson, 1976; Hansen, 1979c; Bae et al., 1994)—likely over hundreds of years—as evidenced by the genetic homogeneity within the infection centers studied (Bae et al., 1994).

Figure 5. Old *Phellinus sulphurascens* sporophore close to the duff on the underside of the stem of a downed, infected Douglas-fir.

There is an interesting contrast of information between the older and more recent literature on occurrence of *P. sulphurascens* sporophores. The early literature states that the fruiting bodies "were not uncommon on the undersides of [400-year-old dead down Douglas-fir] trees" (Buchanan, 1948) and that they "develop abundantly on the underside of logs, uprooted stumps, and occasionally on the trunks of dead, standing" Douglas-firs (Bier & Buckland, 1947). In contrast, literature from the 1970s to 1990s (e.g., Nelson, 1971; Thies & Sturrock, 1995) refers to the relative rarity and/or inconspicuousness of *P. sulphurascens* sporophores, especially in interior stands where conditions are generally

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drier. It could be that sporophore production was more common and therefore more evident/visible in the old-growth stands visited by the early forest pathologists whereas later pathologists tended to work in [much] younger, second-growth stands. This hypothesis is supported by an observation made by Mounce et al. (1940) that *P. sulphurascens* fruiting bodies "are not found as a rule until the wood is in an advanced stage of decay, i.e., on trees that have been dead for some time."

Li (1979) did some work on inducing sporocarps of *P. sulphurascens* in the laboratory on malt agar. Sturrock's team has developed a technique for sporophore induction in *P. sulphurascens* and *P. weirii* (Pellow & Sturrock, 2010); the technique has many potential applications, including induction of fruiting bodies of other *Phellinus* species, many of which are medicinally important (Zhu et al., 2008; Petrova, 2012).

Epidemiology of Laminated Root Rot

Symptoms and signs of LRR may emerge within 5 to 10 years of stand establishment and continue to progress throughout the life of an infected stand (Wallis, 1976b). Infection of new trees and vegetative spread within stands most commonly involves growth of superficial/surface (ectotrophic) mycelia from infected root and stump tissues onto new roots of susceptible hosts (Buckland et al., 1954; Wallis & Reynolds, 1965; Hansen, 1976; Wallis, 1976a, Reynolds & Bloomberg, 1982). The architecture of Douglas-fir (and other conifer) root systems, including rooting depths, plays an important role in disease spread. Sites with shallow soils induce shallow radiating root systems with a high frequency of inter-tree root contacts; these sites tend to have more extensive disease spread and damage than sites with deep soils where roots grow downward and inter-tree root contact is less frequent (Buckland et al., 1954; Reynolds & Bloomberg, 1982; Thies & Sturrock, 1995).

While it is known that *P. sulphurascens* can colonize healthy (i.e., undamaged) bark, and there has been progress on understanding aspects of the infection process using histological and molecular approaches, the precise sequence of events of the infection process has not been fully documented. Once the fungus has penetrated bark tissues, it kills phloem and cambial tissues as it enters and initiates decay in the xylem (Wallis & Reynolds, 1965). Observational evidence suggests that wounding facilitates but is not essential for parasitic colonization by *P. sulphurascens*, and that the fungus will also use points of entry provided by fine roots (G. Reynolds & G. Jensen, retired Canadian Forest Service at the Pacific Forestry Centre, personal communications, May 9, 1988). Barton (1967) found differences in the kinds and amounts of phenolic and non-phenolic compounds when he conducted some preliminary comparative analyses of *P. sulphurascens*-infected and healthy Douglas-fir roots. While there was no consistent pattern of occurrence or concentration for most

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compounds in either type of root, the absence of dihydroquercetin-3'-glucoside and an unknown phenolic extractive in decayed roots and the presence of an unknown, non-phenolic constituent only in healthy tissue may be significant. *Phellinus sulphurascens* is considered a white-rot fungus, i.e., it decays both cellulose and lignin.

The amount of LRR on a site at a given time and its ability to intensify are determined by the initial amount of inoculum, rate of disease spread, existence and distribution of susceptible hosts, and time since first infection (Thies & Sturrock, 1995). Where *P. sulphurascens* is active in maturing coastal forests, diseased trees generally occur in centers around inoculum sources. Dead and dying trees are innermost in the center, crown-symptom trees occur along the radius of the center, and infected but frequently asymptomatic trees occur at the outer margins of the center. Mortality advances 30-35 cm a year (Nelson & Hartman, 1975; McCauley & Cook, 1980). Infected trees generally do not exhibit crown symptoms until *P. sulphurascens* has killed and decayed a major portion (> 50%) of infected roots (Wallis, 1976b). The actual area affected by LRR may be double that revealed based on crown symptoms alone. Wallis & Bloomberg (1981) showed that, while the reliability of establishing the total number of infected trees and area was improved when field crews uncovered and recorded the occurrence of root collar mycelia or stain/decay on stump surfaces, especially for trees 45 to 60 years old, the improvement was not significant enough to warrant the time and funds invested. Thies & Sturrock (1995) and Thies & Nelson (1997) also cautioned that the distribution of LRR over the landscape can be either clumped or diffuse and emphasized the great importance to forest managers that they correctly determine which type of disease distribution they are facing before selecting a LRR management strategy.

Susceptible hosts of any age can be killed by *P. sulphurascens*, although trees infected later in life are better able to tolerate infection (Buchanan, 1948; Thies & Sturrock, 1995; Hansen & Goheen, 2000). Both high- and low-vigor trees are equally susceptible to infection by *P. sulphurascens* (Mounce et al., 1940; Goheen & Hansen, 1994).

Laminated root rot centers tend to be occupied by single clones (genets) of *P. sulphurascens* and, where individual centers meet and coalesce into a larger center, mycelial individuals maintain their separate identities through a somatic incompatibility system (Hansen, 1979b). In this same report, Hansen speculates that the sporadic formation of *P. sulphurascens* fruiting bodies and unpredictable compatibility reactions result from its irregularly binucleate nuclear condition and that these same unpredictable compatibility reactions might demonstrate that basidiospores have more of a role for maintaining genetic variability in long-lived LRR centers by combining with established mycelia than for initiating new infections. The role of basidiospores in epidemiology is important and this needs to be highlighted as an area of needed research.

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Hansen (1986) noted that "genetic differences between clones in mycelial growth rate along roots or in other aspects of pathogenicity could explain observed differences in damage between stands" and tested for this variation using isolates of *P. sulphurascens* from sites differing in LRR severity. He confirmed that such variation exists between clones of the pathogen when he found that two isolates originating from areas of high incidence of infection (18%) were generally more successful at colonizing and growing on roots of inoculated Douglas-fir trees than those from areas of low infection (1%). Sturrock (2005) also found evidence that isolates of *P. sulphurascens* vary in their virulence (the number of trees they kill) and aggressiveness (the rate at which they kill) in 1) a trial that screened Douglas-fir for resistance to two different isolates of *P. sulphurascens* and 2) an inoculation trial that used clonal Douglas-fir. In this latter trial, rooted cuttings (stecklings), representing 23 clones created from nine full-sib families of coastal Douglas-fir, were inoculated with *P. sulphurascens* isolates PFC-Pw581 and -Pw583. Disease development and mortality were monitored for four years. By the end of the experiment, the fungus had killed about 85% and 65% of stecklings inoculated with isolates 581 and 583, respectively (Sturrock, unpublished data).

An important paper by Thies & Nelson (1997) reports that infection status changed for about 39% of Douglas-firs in an Oregon Coast Range stand from observations made a decade apart, and serves to caution us about how we "diagnose" individual trees in and near LRR-affected areas. Eight of 45 trees initially classified as infected based on the occurrence of ectotrophic mycelia on root surfaces were determined to be not infected after 10 years. Two-thirds of trees with reduced crown vigor classified as probably infected due to the presence of *P. sulphurascens* inoculum within 5 m, but with no evidence of ectotrophic mycelia, turned out to be not infected. Finally, about one-third of trees initially considered probably not infected because of their vigorous crowns, no evidence of ectotrophic mycelia, and no inoculum sources within 25 m were determined to be infected 10 years later. Reasons for these "anomalies" between conventionally accepted symptoms of infection and subsequent development of observable disease are not certain, but could include failure of external ectotrophic mycelium to penetrate due to host resistance or poor inoculum potential, proximity to infected trees not always being a reliable indicator of infection, and/or the occurrence of diffuse disease occurrences (single trees) rather than, or in addition, to clumped disease distribution patterns. These results have clear implication for how we survey for, diagnose, and manage LRR.

From observations that individual Douglas-fir trees can respond differently to *P. sulphurascens* infection, Buckland et al. (1954) proposed that trees infected but not killed by the fungus usually had no visible distress symptoms and maintained their vigor by the production of callus tissues and adventitious roots and compensating for loss of their main

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roots. They also stated that *P. sulphurascens* "appears to grow rapidly through the outer portion of the woody core in trees lacking resistance to killing by the disease" and that such trees are completely colonized by the fungus at the time of their death. For Douglas-fir trees showing resistance to killing by *P. sulphurascens*, these same authors describe the fungus as growing "centrally in the woody core" and the heartwood of such trees as having "pronounced bands of resin-impregnated wood" that, among other things, "protects the wood from further saprophytic invasion."

Survival and spread of *P. sulphurascens* and damage attributable to LRR are likely influenced by site conditions, including the obvious soil temperature, pH, and moisture content. As yet no strong evidence exists that any individual site factor (or group of factors) is a reliable predictor of either the presence or intensity of LRR in a given stand (Beale, 1992; Kastner et al., 1994; Thies & Sturrock, 1995). Foresters would like to see more research in this area so that their ability to predict the occurrence of LRR could be improved, especially in light of climate change.

Some research questions remaining in this area include: 1) how above-ground symptoms relate to actual infection on root systems; 2) how disease expression at various stand stages (e.g., free-growing, spacing, and commercial thinning) relates to the actual impact realized in a mature stand; and 3) the best survey protocols to provide optimal decision making concerning LRR for different management decisions such as detection, thinning needs, and pre-harvest planning. With respect to the latter question, early detection of LRR in a stand could guide the management decision of whether the stand should be thinned by PCT, commercially thinned, or marked for early rotational harvest; detection in a pre-harvest survey would guide decisions on what species should be regenerated, recognizing the need to plan 3 years out for resistant stock; and detection at harvest would guide estimates of what yield to expect from a LRR-diseased stand compared to a healthy stand. Each of these management opportunities requires a different kind of survey because of the different management objectives.

Saprophytic Survival of *P. sulphurascens*

After infected trees are harvested, *P. sulphurascens* progressively retreats from the bark surface of infected roots, leaving behind internal (endotrophic) mycelia protected by zone lines, i.e., barriers visible as black lines in cut, decayed wood and commonly formed by several of the higher fungi (Nelson, 1973; Hansen, 1979c; Thies & Sturrock, 1995). Retreat of *P. sulphurascens* tends to progress from the distal portion of infected roots toward the stump (Hansen, 1979c). It is assumed that when *P. sulphurascens* is no longer present on root surfaces, it is no longer infective, i.e., it is shifting from the parasitic to saprophytic phase in its life cycle. However, when Hansen (1979c) described finding ectotrophic myce-

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lia on roots of several 30-year-old stumps only where the roots contacted a rock or the root of a living tree, he suggested that such behavior might indicate that the fungus can re-establish itself on the surface of an infected root by host or contact stimulus. This potential for *P. sulphurascens* to resurface is another potential area of study.

While *P. sulphurascens* can survive saprophytically in undisturbed stumps (Figure 6) and large roots for more than 50 years (Childs, 1963; Wallis & Reynolds, 1965; Hansen, 1976, 1979c), ectotrophic mycelia tend after five or so decades to remain only in discontinuous patches along small portions of parasitically-colonized roots (Hansen, 1979c). This means that stumps from trees harvested decades ago are likely to be less infectious than those more recently harvested (Hansen, 1979c). This point is important to remember as we trend away from old-growth stands to management of second- and even third-growth stands with stumps from trees harvested when only 50 to 60 years old. The longevity of ectotrophic mycelia on hosts other than Douglas-fir and over a range of sites (e.g., biogeoclimatic zones, vegetation types) has not been documented.

Figure 6. Douglas-fir stumps infected by *P. sulphurascens*

Douglas-fir stumps infected by *P. sulphurascens* serve as long-term inoculum that enables the fungus to remain on the site and initiate disease in the replacement stand. The roots of the dead tree seen here came in contact with mycelium on the infected stump and died within a few years.

The succession of organisms in stumps following initial colonization by *P. sulphurascens* through the parasitic phase of its life cycle has not been studied but likely varies over the range of the fungus. There are considerable observational data that the fungus commonly co-occurs with insects (see references on pest complexes in Hadfield et al., 1986) and with *A. ostoyae (= A. solidipes)* in interior Douglas-fir (Cleary et al., 2011), and also that brown cubical rotters and *Trichoderma* spp. often encroach on the zone-line-bounded tissues occupied by the fungus (Hansen, 1979c).

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One likely group of competitors with potential to limit the territory colonized by *P. sulphurascens* through parasitism and hence the amount of substrate available to this pathogen during its saprophytic survival is the endophytes. These are fungi with ability to colonize living tissue but that have little if any ability as parasites beyond using whatever nutrition might be provided as exudates between the cells of colonized tissues. Rather than destroying their host tissue, endophytes live more or less quiescently or commensally in the tissue, apparently at no cost to the host unless it might be through accelerating root senescence. For the most part, endophytes act as opportunists waiting for that tissue to senesce or die, thereby making it possible for them to take possession of the tissue ahead of competitors sitting somewhere on the outside.

As the first to establish in a live Douglas-fir root, endophytes also have the potential to preempt colonization of that root by *P. sulphurascens*, thereby providing a biological control through prevention of infection. In contrast to the difficult task of using other saprophytes to displace *P. sulphurascens* in a stump or roots during its saprophytic existence, prevention of infection by endophytes and other rhizosphere microorganisms may offer the only window of opportunity for biological control of *P. sulphurascens*. Douglas-fir seedlings could be exposed to a consortia of species in nurseries in the same way that mycorrhizal fungi are introduced, thereby introducing them when establishing new stands with nursery seedling. However, it has been shown that nursery seedlings rapidly acquire new mycorrhizal fungi after outplanting. Knowledge of wood endophytes is very limited as distinct from fine root endophytes and only limited studies have been conducted to identify endophytes within woody roots of Douglas-fir (Hoff et al., 2004), but this is one of many areas of research made possible through the use of metagenomic analysis.

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New Approaches to Root Disease Research and Management in the Twenty-First Century

A committee of the National Academy of Sciences, in its report, *A New Biology for the 21st Century*, identified four challenges facing society that have in common the need for greater understanding that will be made possible by the current revolution in the biological sciences. These challenges are food security, ecosystem function in the face of rapid change, sustainable alternatives to fossil fuels, and individualized or personalized medicine. Maintaining and improving forest health is fundamental to maintaining ecosystem functions in the face of rapid change, and forest residuals are potentially part of the solution to a sustainable alternative to fossil fuels.

Over many decades, intensive forest management techniques such as site preparation, nursery production of selected growing stock, planting, and fertilization have greatly increased the production of timber and wood products from Washington's forests. Concurrently, forest land owners with timber management objectives have been sensitive to the potential for LRR to reduce Douglas-fir timber production over long time frames. Understanding the detailed interaction among *Phellinus sulphurascens*, its conifer hosts, and the local biotic and abiotic environments may help to understand the mechanisms that influence host resistance, pathogen virulence and disease expression. Biology for the 21st Century investigations have the potential for important benefits to management objectives ranging from stewardship of basic ecological function to intensive timber production. A new approach to management of LRR depends on the deeper understanding of this disease that is now within reach thanks to new molecular techniques and computing capacity.

Are Altered Disturbance Regimes a Trigger for LRR?

Even though harvesting of Douglas-fir began around 1850, managers were generally not aware that an important endemic pathogen was present until 1940 (Mounce, 1940), and the extent of the problem was not appreciated until the early 1950s (Lawson, 1979). Why did it take 100 years for this currently serious endemic pathogen to reach a significant level of recognition? By 1950, many thousands of hectares of plantations had received about 30 years' exposure to an endemic pathogen under unnatural cutting and regeneration regimes. Two questions arise, neither of which can be answered based on available research: Has LRR increased in severity over the past 100 years, coincident with harvesting and replanting; and could this anthropogenic disturbance have triggered greater LRR activity?

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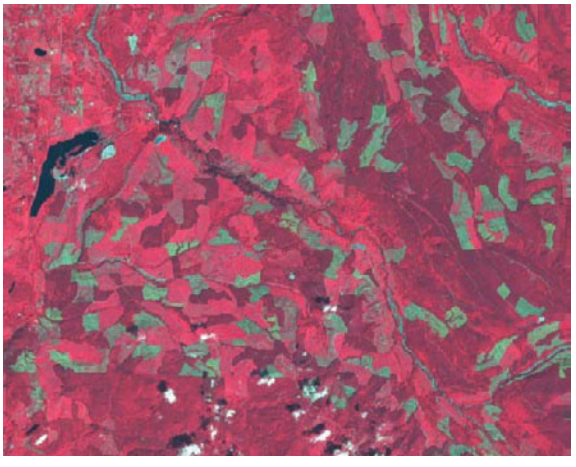
As a general matter, the literature contains no reports on the behavior of LRR in natural stands compared with planted stands or with stands regenerated naturally after management action. Even the latter would involve disturbance because the act of site preparation imposes restrictions on ecological processes. Natural stands should be included in future comparisons.

Figure 7. False-color infrared view of private industrial timberlands northwest of Mt. Rainier National Park.

(Figure 5b in Swanson, 2006; with permission).



Laminated root rot could be triggered by changes in disturbance patterns that influence legacy retention, fire effects, soil disturbances and tree re-establishment. Detritus "carry-over" from one generation to the next is affected by whether a stand was logged, burned or blown down. Logged forests are also fragmented by opening shapes and sizes (Figure 7) that fail to match those characteristic of wild populations of keystone conifers (Douglas-fir in this case) growing in ecosystems dominated by regular return of stand-replacement fire (Agee, 1998).

Exclusion or reduction of fire at the time of regeneration could have many effects, including changing which species would be stimulated to germinate or be killed from the seed bank, thereby altering plant competition and regeneration, affecting the fungi and bacteria that colonize or continue to reside in woody debris and soil, and reducing biochar. Biochar is a byproduct of forest fires (Gavin et al., 2007) that is increasingly recognized as a legacy component vital for ecosystem functions, including resistance of plants to disease (Elad et al., 2011), generally positive influences on beneficial soil microbes (Kolton et al., 2011; Lehmann et al., 2011), absorption of allelopathic compounds (Keech et al., 2005), and potential mitigation of global climate change (Woolf et al., 2010).

Plantations also create deviations from natural stands when nonlocal seedlings are planted; stand density is increased above natural levels from the time of stand establishment to well into the rotation; and heterogeneity in stand composition, age, and pattern is decreased (Figure 7). Shorter establishment times, significant harvest equipment

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impacts, and lower densities of supporting plants, such as nitrogen-fixing alders *(Alnus)* and snowbrush *(Ceanothus velutinus)*, are also critical differences between plantations and natural stands. This is an incomplete list of potential negative impacts related to effects at establishment. Intermediate treatments in managed forests are also outside natural stand development patterns and could be interfering with the reestablishment of normal ecological networks, for example, through production of fresh, living stumps inhabited by root pathogens early in the rotation, or episodes of soil compaction during commercial and pre-commercial thinning.

A New Community Ecology

Changes at the time of stand establishment and during intermediate silvicultural treatments can be expected to have had significant effects on Douglas-fir-dominated ecosystems. Thus, we need to gain a better understanding of the effect of detrimental levels of LRR in Douglas-fir ecosystems and their complex causes over time on ecosystem functions, processes, and patterns. Recent revolutions in the theory of community ecology ["the study of patterns in the diversity, abundance, and composition of species in communities and of the processes underlying these patterns" (Vellend, 2010, p. 183)] provide an approach. This transformation is built on the new recognition of the roles of intraspecific variation, interspecific interaction, and community adaptations. These new ideas are fundamental to the mission of this report because forest management is "applied community ecology." Intraspecific variation is important to pest management, genomic approaches, ecological networking, and plantation forestry. The myriad of interspecific interactions within the host-pathogen microbiome is an example that surely must influence disease expression.

The above ideas are incorporated and extended under the new and rapidly developing discipline of community genetics (Whitham et al., 2003, 2006). This approach combines ecology and evolutionary genetics to focus on how intraspecific variation present in foundation (keystone) species impacts functional relationships within particular ecosystems (Genung et al., 2012; Whitham et al., 2012). Community genetics is well suited to the application of modern molecular techniques (Whitham et al., 2008; Wymore et al., 2011).

The new field of community ecology is built on the concept that "local adaptation" (Kawecki & Ebert, 2004, p. 1225) is a common feature of wild populations. Divergent selection (Feder et al., 2012, p. 342) and "epigenetic inheritance" (Uller, 2008, p. 432) combine to form intraspecific "demes" (Whitham et al., 2012, p. 271) adapted to specific niches. Community ecology is a functional amalgamation of ecology and microevolutionary selection for adaptation at fine scales present within stands and landscapes acting on intraspecific variation at the species, interspecific, and community levels.

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Recognition of the importance of intraspecific variation to ecological adaptation presents an important quandary to current silvicultural practice because most forest regeneration decisions are based on the assumption that gene flow following the last ice age will have homogenized fitness over areas hundreds to thousands of acres in size. However, the key idea that modern community ecology brings to our understanding of the forested landscape is that local adaptation happens in current time and place on a scale of tenths to hundreds of acres. This impacts the important question of how to manage and at what scale (Funk et al., 2012).

Demes or "ecotypes" (Hufford & Mazer, 2003, p. 147) must be demonstrated to prove their reality and to define them. Such demonstrations can be based on tests of responses or performance in "home turf" vs. "foreign turf". The most common technique for testing samples of a species' local populations for adaptation in home and foreign environments is to construct "reaction norms" (Hunt et al., 2004, p. 329). Samples are chosen to represent several sub-populations at different sites. Responses are observed and plotted after the samples have been grown under a broad set of home and foreign comparisons (Kawecki & Ebert, 2004; Leimu & Fischer, 2008). When lower fitness occurs at a "non-home" site, a "home site advantage" and locally adapted deme are indicated.

As with any biological analysis, protocols must be followed in order to obtain valid interpretations. One problem for forest trees is that the way seedlings are grown prior to an experiment can alter their physiology. Growing seedlings in a uniform, idealized nursery environment could have a potential and lasting phenotype-modifying effect. Although the discussion from reaction norms that follows can provide evidence of the utility of studying intraspecific variation in organisms—tree and pathogen species alike—it also indicates the greater need and role for molecular techniques that elucidate activity of specific genes and gene suites under different biotic and abiotic environments to provide the means to preserve or use existing adaptive genetic variation.

How Big a Deal is Local Adaptation in Douglas-Fir?

To date, few studies of local variation in tree populations have been published (Savolainen et al., 2007). An altitudinal reciprocal transplant study of Sakhalin fir in Japan was installed in 1974 and measured for 36 years (Ishizuka & Goto, 2011). This study was conducted in accordance with accepted criteria and clearly demonstrated local adaptation in the form of home-site advantage in a conifer species. Complicated and expensive transplant studies, such as the above example, may not be required to map and understand the role of local adaptation in Douglas-fir forests, since new molecular techniques can reveal the "footprints" of adaptation in genomes (Ellison et al., 2011; Grivet et al., 2011; Stapley et al., 2010; Funk et al., 2012; Lowry, 2012a; Slavov et al., 2012).

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Accumulating evidence indicates that local adaptation is a fundamental feature of all life forms, and the biology underlying speciation with gene flow is becoming apparent (Bennington et al., 2012; Lowry, 2012b). A recent review of epigenetic regulation in species of forest trees supports this conclusion (Brautigam et al., 2013). Given that post "Modern Synthesis" (Bird et al., 2012) evolutionary biology suspends the idea that strong gene-flow will block local adaptation, the role of local adaptation in forest management can no longer be ignored (Laikre et al., 2010; Nosil & Feder, 2012; Slavov et al., 2012).

Has Local Adaptation Occurred in *Phellinus*?

Much of the case for local adaptation and community interaction for the host presented above also holds for the pathogen. Responses to differences in culture media and temperature gradients illustrate the potential importance of local adaptation as a consideration in management of LRR. The *Phellinus* literature includes appropriate data for analysis using reaction norms (Trappe et al., 1973; Nelson & Fay, 1975). These methods could be applied to various physiological responses of Douglas-fir related to resistance or susceptibility to LRR, but have not been widely applied to the study of abiotic adaptation of fungi or their abilities to overcome host exclusion mechanisms.

Trait Functions: Understanding the Mechanics of Community Evolution

Reaction norms (genetic performance across an environmental gradient) supply useful interpretations of the interactions among evolutionary forces, biotic communities and the environment for all sorts of traits (see McDonald et al., 2005; Nicotra et al., 2010; Lowry, 2012a). Characterists of thermal performance curves (thermal reaction norms) are widely used in the analysis of thermal adaptation (Stinchcombe et al., 2012, Angilletta, 2006; Angilletta et al., 2010; Boyles et al., 2011; Thomas & Blanford, 2003). Such methods have not been widely applied to the study of forest ecosystems, even though they have high potential to unlock key aspects of ecoevolutionary mechanics in complex natural systems. The above methods were used to analyze net photosynthesis and other functional traits in plants (see Angilletta, et al., 2010; Cunningham & Read, 2003).

Resistance or Tolerance

The ability of plants to muster defenses against pathogens and insect pests, or at least tolerate their insults, is the norm and not the exception in nature. Indeed, the establishment of a successful host/pathogen relationship is rare among the myriad of microorganisms associated with plants, both culturable and unculturable. Research done mainly on angiosperms shows that the defenses to would-be pathogens are both local, by way of production of phenolics and antibiotic-like substances known as phytoalexins, and systemic as pathogenesis-related (PR) proteins and other changes in response to signaling molecules

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(Heil & Bostock, 2002). Two signaling molecules have been shown to play major roles in plant defenses: salicylic acid, responsible for systemic acquired resistance or SAR (van Loon et al., 1998), also known as induced systemic resistance (ISR) (Heil & Bostock, 2002), and the jasmonic acid signaling cascade responsible for defense against herbivorous insects (Ryan & Jagendorf, 1995) and some pathogens (Vijayan et al., 1998). SAR induced by nonpathogenic rhizobacteria has been shown to confer protection against subsequent attack by pathogens (van Loon et al., 1998). This probably also happens in response to endophytes and mychorrhizae, although little research has been done with these plant-associated microorganisms and even less relative to trees.

Returning to the discussion above of local adaptation in current time, and the occurrence of demes or ecotypes, induced systemic resistance triggered by root-associated microorganisms associated with certain soils or aspects of the landscape could account for apparent differences otherwise attributable to local adaption.

Recommendations on the potential to manage LRR through tree breeding for enhanced genetically based resistance of Douglas-fir to LRR and an analysis of the rhizosphere microbiome of Douglas-fir at different stages of the host-pathogen interaction using metagenomics and other modern molecular methods are given in a subsequent section. In addition, the reaction norms discussed above provide a basis to better understand the tolerance/resistance tradeoff found in infectious diseases (Baucom & de Roode, 2011; Kause & Odegard, 2012; Roux et al. 2010). This approach also facilitates the separation of phenotypic variance into its constituent parts (Kause et al., 2012). Application of reaction-norm analysis has received some attention in plant pathology (i.e., Inglese & Paul, 2006; McDonald et al., 2005) but holds great promise for identifying types of host-pathogen specializations to enable the use of modern molecular methods for better management of forest pests and diseases.

Answers in Community Genomes: Networks and Community Genetics

Sequencing the human genome and over 100 genomes of other organisms has generated a remarkable new view of the biosphere. The initial view of the DNA between protein-coding genes as "junk DNA" has been transformed (Pennisi, 2012a) to recognize its role as actively participating in a rich array of gene regulatory networks (Maurano et al., 2012) that translate biotic and abiotic signals into physiological and evolutionary action (Zhang et al., 2013; Weber & Agrawal, 2012). These regulatory networks contribute to creating locally adapted populations that can undergo rapid response to changing conditions (Hare, 2012) and fuel transgenerational epigenetic adjustments to local conditions (Uller, 2008). Experiments in plants show that related seedlings planted in local "home soil" grow better (Smith et al., 2012) and support more robust mycorrhizal associations (File et al., 2012) than intraspecific non-local demes.

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Such interspecific interactions may lead to cooperative evolution in biotic interactions (Cordero et al., 2012; van Veelen et al., 2012). Consequently, we should not be surprised to see complex ecological networks (see Proulx, 2005; May, 2006) guiding behavior of ecological communities in oceans, waterways, and on land (Lewinsohn & Cagnola, 2012)—and now host-pathogen systems (Moslonka-Lefebvre et al., 2011). In general, we now expect that the microbiome (totality of all microbes and their genomes in a particular ecological setting, i.e., the rhizosphere), will show profound interactions with resident plants (Simard et al., 2012; Schnitzer et al., 2011), animals (Ezenwa et al., 2012), and fungi, including endophytes (Sessitsch et al., 2012), arbuscular mycorrhizal (Montesinos-Navarro et al. 2012) and ectomycorrhizal associations of Douglas-fir (Beiler et al., 2009). The fundamental importance of a soil microbiome is at least partially understood in agriculture (Berendsen et al., 2012) and merits greater study in forestry.

Network theory (Newman, 2012; Fontaine, 2011; Bascompte, 2009, 2010), i.e., the analysis of representations of all pairwise interactions among organisms in environments, reveals the architecture of local biological interactions. A fusion of ecology, genomics, and network theory has created the new discipline "ecophylogenetics" (Mouquet et al., 2012; Weber & Agrawal, 2012). This new discipline reveals the potential for intraspecific population subdivisions in all network members that play potentially significant roles in the response of forest communities to disturbances, as was discussed above on community ecology. An important component of intraspecies subdivisions is the concept of conservation units (taxonomic or population units significant enough to need to be retained to preserve function), as discussed above (Funk et al., 2012). On the one hand, we must look beyond "species" and, on the other hand, realize that the new biology fosters a more complete ecological understanding of the living world (Ostfeld et al., 2008; Borer et al., 2011; Magurran & McGill, 2011).

An example of the use of network analysis for a pathogen in different settings is found for *Phytophthora ramorum*. This oomycete is a well-known pathogen of trees and other woody species in coastal northern California and Oregon and is the cause of Sudden Oak Death. It is variously a bole and leaf pathogen on its many different tree and shrubby hosts. It is a severe bole pathogen on coast live oak and tanoak, but it is as a leaf pathogen on bay laurel, Oregon myrtle and Rhododendron hosts that it gains its greatest potential to produce sporangia and spread across landscapes. Network analysis has been used to contrast its relationships in nursery vs. forest settings in Great Britain (Moslonka-Lefebvre et al., 2011).

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A New Forest Management Paradigm

Loss of local adaptation or interaction with maladaptive phenotypes could create network malfunction such that nonlocal x local crosses could cause outbreeding depression and future complications (Ouborg et al., 2010). Shapes, sizes, and edge characteristics of stands all impact the microclimate of planting openings. Such abiotic variations could impact niche relationships of species pairs within ecological networks. Impacts of changing detritus legacy are not well understood but could be important. Reconstruction of how natural stands were established over time using dendrochronology of stems clearly shows that, in the past, natural stands were established over longer periods of time, at lower initial stocking densities, with higher species diversity and more heterogeneous growth patterns than are now found in plantations (Franklin & Hemstrom, 1981; Tappeiner et al., 1997; Winter et al., 2002). Moreover, actinorhizal (nitrogen fixing, e.g. alder) shrubs and trees represent an important biological legacy in most natural Douglas-fir stands (Hibbs & Cromack, 1990; Jeong & Myrold, 2001).

Returning to the question of forest disturbance, thinning is an intermediate treatment perceived to increase LRR impacts. Is disease expression actually increasing, as has been observed in case studies of *Armillaria* root disease (Hood et al., 1991; Kile et al., 1991). Is it simply related to increased human attentiveness to stands that have received a management investment? Could it be due to increased pathogen aggressiveness, decreased tree vigor, or changes to some unknown component of an existing ecosystem? Is thinning simply leaving the standing infected trees more vulnerable to windthrow (Lawson, 1979). The effects of thinning on LRR have not been thoroughly evaluated in the Pacific Northwest and should be examined using both traditional forestry measurements and intensive evaluations of biochemical interactions among host, pathogen and other interacting components of the microbiome.

Similarly, little is understood about how increased length of a rotation might interact with LRR or any other combination of species with trophic or biotic connections in the ecosystem. It is abundantly clear that, for *A New Biology for the 21st Century* to be applied to forest management, much more attention must be given the environmental definition of stands or communities and to actually measure both the biotic and abiotic vectors of influence.

Mapping Relevant Local Adaptation

In forestry, management units are labeled as "stands," but these usually are not defined by rigorously following the patterns of significant ecological features on landscapes. The long-term battle over assembly of plant communities, randomly (as a continuum) or nonrandomly (in discrete and characteristic patches), continues to this day. It is evident that

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community ecologists are moving toward sufficient understanding to reliably map niche boundaries (patches) on the landscape (Dray et al., 2012; Austin, 2002; Götzenberger et al., 2012; Guisan & Zimmermann, 2000; Heegaard, 2002). Some studies include efforts to understand microbial interactions (Bever et al., 2010). Recent developments in this discussion elevate the potential value of plant community classification to benefit forest managers. A system developed for management of *Armillaria* root disease in the western United States (McDonald et al., 2000) could serve as a model for LRR.

Management of LRR and Ecosystem Services

Another aspect of *A New Biology for the 21st Century* is the concept of ecosystem services. Recent papers discuss ecosystem services from the point of view of plant pathology (Cheatham et al., 2009) and from the larger viewpoint of the landscape (Kinzig et al., 2011). The State of Washington recently released a report on ocean acidification (Washington State Blue Ribbon Panel on Ocean Acidification, 2012). Carbon dioxide and nitrous oxides are important contributors to acidification that can be mitigated through application of appropriate forest practices. Biochar is a replacement for an important forest ecosystem constituent created by forest fires and, as an attractive option for the future, has received much attention lately (Woolf et al., 2010; Post et al., 2012). Longer rotations can enhance the ability of forests to sequester carbon (McKinley et al., 2011) and may have associated positive benefits by enhancing the recovery time following disturbances (Franklin et al., 2007). We can employ new theories such as community genetics, ecological network analysis, and niche construction, as well as new genetic techniques such as high-throughput sequencing, genomics, metagenomics, and metatranscriptomics to help investigate and solve the problems of ecosystem instability and excessive LRR expression.

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Contemporary Management of Laminated Root Rot: Strategies and Policies

The current and future societal demands for wood and paper products and ecological services, being placed on a shrinking resource (managed forests), will intensify management by the DNR on state-held lands. Losses such as those ascribed to LRR and other pests cannot go unaddressed. There is much that can and, in many cases, is being done now to manage LRR in Washington forests generally and in DNR-managed forests more specifically. Moreover, as new information becomes available, such as the availability of molecular techniques for more rapid and accurate diagnosis of the disease, these can be readily integrated with current practices. The examples below review the status of current management practices somewhat in order of their importance.

Monitoring and Modeling the Location, Rate of Spread, and Economic Importance of LRR

Fundamental to any method of management is to know accurately the location of LRR infection centers within stands and on the landscape. Knowledge of the location and/or severity of LRR centers could inform decisions to accelerate harvest plans to preempt future mortality, cut around disease pockets, and create non-host tree buffers to restrict spread of the disease. Practices intended to starve the pathogen will limit its ability to infect in the future.

On the other hand, it is important to avoid thinning or partial cutting in and around infection centers because many trees in close proximity to infection centers, even those with green crowns, will likely have extensive root rot and be subject to windthrow if the stand is thinned. Knowledge of LRR infection centers and their clustered or diffuse nature can cause a manager to delay a partial cut in order to make a single regeneration cut that will extract more value of the resource and allow regeneration of more shade-intolerant, resistant species. The investment in uninformed practices is unlikely to be recovered. Indeed, the DNR may need to accept significant shortfall in timber production if it embraces alternative non-timber benefits on some severely infected sites and focus disease management on their highest productivity sites with the highest potential for valuable timber production.

Suitable and accurate ground-based survey techniques have been developed (e.g., Bloomberg et al., 1980; Hadfield et al., 1986; Morrison et al., 1992; British Columbia Ministry of Forests, 1995; Thies & Sturrock, 1995), but there is an unmet and ongoing need for proper

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training of personnel conducting root disease and/or other forest health/condition surveys. There is also a need for more research to improve the accuracy and cost-effectiveness of ground-based LRR surveys.

Detection of LRR-affected stands and trees using aerial photography and remote sensing has been successful (e.g., Johnson & Wear, 1975; Williams & Leaphart, 1978; Wallis & Lee, 1984; Thomson et al., 1996) but is only infrequently used operationally. Recent research has focused on the use of compact airborne spectrographic imager (CASI) data for detecting LRR (Leckie et al., 2004).

Over the past two decades, progress has been made to incorporate the effects of LRR and other root diseases into growth and yield models. Tree growth models such as the Forest Vegetation Simulator [(FVS) (U.S. Forest Service, 2012)] have been extended to include submodels dealing with root disease, including LRR, such as the Western Root Disease Model (WRDM) and the linked Root Rot Simulator (Rotsim) and Tree and Stand Simulator (TASS) models (Frankel, 1998). Root disease dynamics are modeled in six major parts:

- Size and distribution of root disease centers at the start of the simulation;
- Dynamics of tree infection inside centers;
- Tree mortality and growth inside the centers;
- Spread and enlargement of centers;
- Persistence of root disease following tree harvest, through a newly regenerated stand; and
- Interaction of "other agents" (windthrow and four types of bark beetles).

Information on variables such as stand structure, site conditions, and inoculum condition are entered as either keywords or numeric inputs. Based on host presence and stand information, the models then calculate the rate of spread of the fungus and the number of trees likely to become infected. The models can simulate the combined effects of root disease, occurrence of windthrow, or management activities like stumping that may reduce inoculum in future stands. The WRDM can also simulate the combined effects of root disease and bark beetle attack (Thies & Sturrock, 1995; Hansen & Goheen, 2000). Work with TASS/Rotsim on simulating root disease in stands with two or more coniferous species is ongoing, which provides the advantage of updating the models as new information becomes available. One area in which WRDM extension may be improved is to incorporate different rates of mortality for different periods in the life of stand cohorts of resistant vs. susceptible species at different tree ages. For example, pine and larch are resistant to LRR and are normally considered moderately resistant to other root diseases, but can succumb

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to *Armillaria* root disease during only the first several decades of their lives. However, their rates of mortality to root disease in the North Idaho model extension are currently set to a constant low level throughout their development. Model inputs may be improved as additional data from long term plot networks become available.

New techniques have been developed for pinpointing individual diseased trees and disease centers using aerial photography, computer-assisted technology, LiDAR, and global positioning systems (GPS). New knowledge about the behavior of LRR is incorporated into these models as it becomes available. However, links between researchers and forest managers, and operational use of models for and by forest managers, could/should be improved.

In addition to simulation models, economic models also can be used. For example, after conducting a survey of *P. sulphurascens* in a Douglas-fir stand, an economic analysis can be done using present net-worth analysis for predicting return on investment (Russell et al., 1986). Costs of site preparation and stump removal, planting, thinning, vegetation control, and fertilization can be included, and volume/acre (or hectare) can be predicted with and without LRR. FVS also currently has ability to calculate volume and board feet, and potential losses to fire through its fuels and fire modeling extensions.

Replanting Infested Sites with Nonsusceptible Trees

Control of soilborne plant pathogens in agricultural systems commonly involves rotating the crops, thereby allowing time for the pathogen(s) of one crop, e.g., a grass crop, to die out while growing a different crop, e.g., a broadleaf crop, that is less or not susceptible to the pathogen(s) of the grass crop. In forestry, "rotation" means successive generations of trees, including the same species of tree, such as the valued Douglas-fir, as has occurred naturally for thousands of years. Unlike agriculture, stands can have uneven-aged structure, with multiple cohorts of the same or different species established at different times. In fire-prone ecosystems, these cohorts correspond with fire return events. In moist forests with long fire-return intervals, root disease can create the gaps that allow younger cohorts of shade-tolerant species to establish, including Douglas-fir. Partial cutting to provide seed trees or shelter wood cuts to reduce seedling stress during seedling establishment automatically creates a multiple-cohort situation. Understandably, it is not always feasible to treat forest root diseases as one would a root disease of an agricultural crop. E. M. Hansen (personal communication) showed in northwest Oregon that a 30-year rotation of red alder or cottonwood following a second-growth Douglas-fir regime reduced *Phellinus* inoculum potential, but viable inoculum persists on the site today. Nevertheless, planting of species not susceptible to *P. sulphurascens* (e.g., Filip & Schmitt, 1979; Nelson & Sturrock, 1993) is a practice well accepted by foresters.

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One problem is that surveys for the occurrence and severity of LRR in a stand are often decoupled from traditional regeneration decisions, because it takes a minimum of two to three years to produce an alternative seedling crop with the correct seed source/genetics to otherwise avoid replanting of susceptible species. Thus, that many infested sites get planted back to Douglas-fir can be due to the lack of regeneration stock. Moving these LRR-management decisions forward to the pre-harvest phase allows time for nursery staff to plan for production needs.

Practices to Lower the Infection Potential for New Plantings

The efficacy of stump removal (stumping) (Figure 8) and push-felling in reducing *P. sulphurascens* inoculum also has been tested (e.g., Arnold, 1981; Thies & Nelson, 1988; Bloomberg & Reynolds, 1988; Morrison et al., 1988; Sturrock et al., 1994; Sturrock, 2000; Thies & Westlind, 2005; Cleary et al., 2013; Sturrock, 2012). While the long-term efficacy of stumping is still a matter of discussion among and between forest pathologists and forest managers, the major concern still outstanding on inoculum reduction is the balance between costs and benefits of the treatment. Stumping requires very heavy machinery, causes considerable soil disturbance and is limited to slopes less than 30%, leaving many forest sites unavailable to this treatment. Stump removal does not eliminate the problem but does lessen mortality in the next rotation. Sediment flow caused by stumping in some instances might endanger streams listed for salmon recovery.

Figure 8. Laminated root rot management using a backhoe and removal of infected stumps.

Stumps are typically turned over and left on the soil surface to dry out. The fungus in the stumps eventually dies and cannot infect new trees. However, the fungus is not eliminated from the site because it can survive in the soil in broken root fragments and infect new trees.

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Chemical inactivation of *P. sulphurascens* inoculum with fumigants such as chloropicrin has been demonstrated (Thies & Nelson, 1982, 1987, 1994; Fraser et al., 1995), but fumigants do not penetrate sufficiently into buried roots to adequately eliminate this pathogen. Currently, fumigation is not used to treat stumps. Moreover, because of cost and regulatory policies that restrict the use of pesticides in forests, fumigation will likely remain limited to special situations, such as the control of tree seedling pathogens in forest-tree nurseries.

An approach known in BC as "bridge tree removal" relies on the removal of susceptible but asymptomatic hosts around disease centers in maturing stands to restrict the spread of *P. sulphurascens*. This leaves standing dead and dying trees for wildlife habitat (Figure 9). This procedure, first published by Childs (1955) and recommended by Wallis (1976b) and Hadfield (1985), is based on the concept that *P. sulphurascens* centers can be isolated by harvesting "bridge trees" at the boundaries of infection centers. A bridge tree can be defined as any *P. sulphurascens*-susceptible tree that is likely to have root contact(s) that can bridge between infected portions of a stand and asymptomatic portions of the stand. Preliminary work by the Sturrock team using its inoculation technique (Sturrock & Reynolds, 1998) indicated that mycelia of *P. sulphurascens* can still be successfully established on roots of 1- to 5-year-old Douglas-fir stumps, although continued growth and longevity of the transferred mycelia were not determined (unpublished data). The bridge tree removal concept could be investigated more fully, in part to determine whether establishment success can be related to prolonged stump survival due to root grafting with neighboring trees, such as is indicated by callus growth that can sometimes completely seal over the surfaces of cut stumps. In such a case, it could be argued that cutting of certain bridge trees could provide the LRR pathogen with root systems that continue to survive in a weakened condition less able to defend against colonization by *P. sulphurascens*.

Figure 9. Laminated root rot management by harvesting around root disease pockets and leaving standing dead and dying trees for wildlife habitat.

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Molecular Biological Approaches to Understanding and Managing Laminated Root Rot of Douglas-Fir

Traditional forest pathology research is changing because of the demographics of current researchers (retiring) and shifts in funding priorities in research organizations (universities, U.S. Forest Service, Natural Resources Canada) away from plant pathology. As with other areas of biological and environmental research, there is a shift toward greater use of molecular approaches and multidisciplinary teams. Powerful genetic technologies are emerging that allow novel, integrative approaches to better understand the dynamics of the host-pathogen interaction and the multiple interacting components of forest ecosystems that contribute to forest health or susceptibility of individual forest trees and species to root diseases. Below are some examples, presented somewhat in priority order.

Population Genetics, Phylogeography, and Phylogenetics of the Pathogen

The means to study the genetics and genetic structure of the pathogen at the population level using restriction fragment length polymorphisms (RFLPs) and genetic markers has opened an entirely new approach to understanding a pathogen's genetic diversity, evolutionary biology, potential geographic origin and global movement, phylogenetic and evolutionary relationships with other *Phellinus* species, such as *P. weirii*, and whether its reproduction is sexual or asexual. An example is found in the basidiomycete fungus *Rhizoctonia solani* anastomosis group (AG)-1 IA, which is a major pathogen of many agricultural crops worldwide and a functional diploid. Ciampi et al. (2008) used 10 microsatellite loci to investigate the genetic diversity and population structure of 232 isolates of this basidiomycete fungus from five soybean fields in Brazil. Most of the multilocus genotypes represented among the 232 isolates were geographically site-specific, indicating a low genotypic diversity. This result combined with a low Hardey-Weinberg equilibrium (test for the contribution of sexual recombination to genetic structure) and a high degree of population subdivision led to the conclusion that this population of *R. solani* anastomosis group (AG)-1 IA on soybeans in Brazil depends predominantly on asexual reproduction, short-distance dispersal of vegetative propagules (mycelium or sclerotia), and limited long-distance dispersal, possibly via contaminated seed. As another example, Zaffarano et al. (2009) characterized 500 isolates of the barley scald pathogen *Rhynchosporium secalis* with RFLPs for two nuclear DNA sequences together with the mating-type idiomorphs to estimate putative migration patterns and the demographic history of this pathogen. The 500 isolates of the pathogen represented 60 field populations and five continents. Their results indicated that the pathogen had only recently migrated out of northern Europe, its apparent center of origin, establishing reproductively isolated founder populations in other regions of the world where barley is grown.

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Genetic variation among and within isolates of *P. sulphurascens* has also been investigated using RFLP analysis (Bae et al., 1994). This research demonstrated that infection centers are initiated by basidiospores and that subsequent basidiospore-initiated immigrations are relatively rare events. The role of basidiospores in the spread of *P. sulphurascens* has been "queried" by several researchers in the past 70 or so years, and most have concluded that their data support the thinking/observations that vegetative (clonal) rather than spore spread is the most common mode for the fungus. One paper that concluded differently is that by Dickman & Cook (1989). This study looked at fire and *Phellinus* over a very large study area and over a time period of 1,300+ years. Their detailed analyses of several aspects of disease and fire, including center ages, genotypes, distribution, size, etc., led them to several interesting conclusions, including the following:

"The existence of 44 genets in a sample of 61 infestations from an area of 3900 ha, the dominance of young genets that consist of single infestations having simple circular outlines, and the improbable dispersion of infestations of the various classes of genets suggest to us the establishment or modification of genets from basidiospores. We fail to find evidence in this distribution that the dominant mode of establishment of infection centers is vegetative, although it certainly plays an important part (and the vast majority of trees are almost certainly infected vegetatively)."

Recently, Leal et al. (manuscript in preparation) carried out a comparative genetic diversity study among *P. sulphurascens* isolates from western North America (Canada and United States) and Eurasia (Siberia and Japan) to investigate the population genetic structure relative to the origin of this species (i.e., introduced vs. native). Analysis of several small and large mitochondrial ribosomal RNA subunit genes [(mtSSU rRNA and mtLSU rRNA) and six nuclear loci [internal transcribed spacer region (ITS), actin (ACT2), RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2), putative laccase (LAC-like), and translation elongation factor 1-alpha (TEF1)], revealed that none of the alleles were shared between the western North American and Eurasian populations of *P. sulphurascens*. This work shows that *P. sulphurascens* in Eurasia is highly diverse, indicating that Eurasia could be the center of origin of this fungus, but that gene flow has a long history of being restricted between these two continental regions.

Metagenomics and Transcriptomics

Ever newer and more advanced DNA and RNA mass sequencing techniques are allowing researchers to quickly and inexpensively identify all of the transcribed genes (the transcriptome) specifically involved in a host/pathogen interaction, to identify both culturable and non-culturable microorganisms, including down to genus and even species, by mass sequencing part or all of their collective genomes (metagenome), or even to identify what

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genes are transcribed in a metagenome (metatranscriptome). These techniques are rapidly supplementing and even replacing the traditional role of isolating and culturing bacteria and fungi from their natural habitats or observing them microscopically, while providing a more complete picture of microbial populations and their interactions and functions under natural conditions.

By comparing metagenomic and metatranscriptomic data for forest soils, Stewart et al. (2010) found a positive relationship between gene expression level and DNA sequence conservation. This relationship shows the functionality of meta-transcriptomic analyses to answer questions of taxonomy and functional gene-expression samples across multiple soil types, locations, and disease severity levels.

These methodologies are already being used to understand host-pathogen interactions of several forest root diseases. The greatest advances have been in several root diseases within the *Heterobasidion* root disease complex. Sun et al. (2011) used transcript profiling to elucidate mechanisms of biological control of the European pathogen of Scots pine (*Pinus sylvestris* L.), *Heterobasidion annosum*, by the saprophytic *Phlebiopsis gigantea* when applied preemptively to the freshly cut surfaces of stumps. Total RNA was extracted from phloem and xylem tissue at different times up to eight weeks after 10-year-old seedlings of Scots pine were challenge-inoculated with either *P. gigantea* or *H. annosum*, wounded, or left untreated. Following standard protocols, the RNA extracted from the phloem tissue representing the four respective treatments was reverse-transcribed into double-stranded cDNA, and these four cDNA samples were then used to construct cDNA libraries suitable for sequencing. Sequencing of the cDNA contigs representing the samples challenge inoculated with either *H. annosum* or *P. gigantea* revealed elevated transcript levels of several genes important in host-defense responses to pathogens, including genes for lignification, programmed cell death, and jasmonic acid signaling. Transcripts identified with programmed cell death also appeared in response to wounding.

In combination with histochemical studies that revealed an increased, if somewhat ephemeral, level of phenolics and lignified cells in wood challenged-inoculated by *P. gigantea*, these results clearly indicate that biological control of *H. annosum* by prior inoculation of freshly cut pine stumps with *P. gigantea* is due in part to induced resistance of the living wood to *H. annosum*. Possibly the defense response triggered by *P. gigantea* prior to the arrival of spores of *H. annosum*, itself now relegated to the role of a secondary colonist, precludes mechanisms *H. annosum* might normally use to avoid or escape host defense responses it might have triggered as the primary colonist.

In a study of host-parasite interactions of another related European *Heterobasidion* species, Arnerup et al. (2011) compared the transcriptional responses in bark of Norway

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spruce to *H. parviporum* infection and wounding, respectively, using cDNA-amplified fragment length polymorphisms. The expression patterns as recovered transcript-derived fragments (TDFs) were similar for the two treatments, except for a slightly enhanced reaction in response to inoculation with the pathogen. Transcripts identified as jasmonic acid (JA)- and ethylene (ET)-mediated signaling defense pathways were among those that accumulated simultaneously in response to both treatments, while accumulation of transcripts identified with genes related to systemic acquired resistance (PR1) accumulated after infection by *H. parviporum* but not in response to wounding. In a separate study that included inoculation of Norway spruce with *P. gigantea* as a third treatment, these investigators report the sequences of three 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase genes identified, respectively, as PaDAHP1, PaDAHP2 and PaDAHP3, and their differential expression in response to infection with either *H. parviporum* or *P. gigantea*. These results give a clear indication of the central molecular mechanisms involved in the wound response and induced defenses in Norway spruce.

Transcriptome studies of *Armillaria* root disease pathogens have also been initiated (Ross-Davis et al., 2013). In a study based on a large genet that was infecting numerous hosts of multiple woody plant species, 20,882 expressed genes of *A. solidipes* were identified from a mycelial infection fan on grand fir (*Abies grandis*). Within this *A. solidipes* transcriptome, interest was focused on genes with ascribed functions relating to pathogenesis, host substrate utilization, and response to post-infection host environment. Such studies contribute to the growing body of knowledge on genes associated with pathogenesis and other metabolic functions within diverse fungal pathogens that cause root disease of forest trees.

Transcriptome studies could be used to compare and contrast gene expression in the Douglas-fir/*P. sulphurascens* pathosystem during the parasitic and saprophytic stages of the life cycle, respectively. The Sturrock team already has several transcriptomes created in 2011 from healthy, *P. sulphurascens*-challenged, and drought-stressed Douglas-fir seedlings. In a recent study, putative defense- and/or stress-related genes were identified from Douglas-fir that was infected by *P. sulphurascens*, and putative pathogenis- and/or stress-related genes were identified from the *P. sulphurascens* that was infecting Douglas-fir (Islam et al., 2013). Furthermore, time-course studies demonstrated changes in expression levels of defense-related genes in Douglas-fir during the infection process. Such information provides a critical framework for understanding host resistance and pathogen virulence in the LRR pathosystem.

Metagenomics, on the other hand, could help deduce ecological functions of the critical biotic components, including host/pathogen/interacting microbes in forest ecosystems under diverse environments with variable impacts of root disease. Other approaches, such as community genomics/metagenomics and community transcriptomics/metatranscrip-

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tomics, may shed new light on the microbial communities associated with forest ecosystems as well as their ecological function. Such molecular genetic studies will help identify key genetic interactions that characterize stable (healthy) versus root-disease-impacted forest ecosystems. These approaches are perhaps best applied to a model system of Douglas-fir and root diseases, where sufficient baseline information is available to facilitate these studies and help interpret the results.

Sessitsch et al. (2012) did a metagenomic analysis of the bacterial endophytes in roots of field-grown rice plants. From this analysis, they were able to predict traits and metabolic processes based on putative functions deduced from protein domains or similarity analysis of protein-encoding gene fragments. They concluded that this endophytic community had a "high potential" for plant-growth promotion, remediation of plant stress, biological control of pathogens, and might even be involved in N_2-fixation in the endorhizosphere. Results such as these reveal that there is so much more going on in roots and the root environment than could ever be revealed by conventional culture and microscopic techniques of the past.

Deciphering the Microbiome of the Douglas-Fir Rhizosphere

There has been little to no research and/or documentation on how *P. sulphurascens* interacts with other organisms such as endophytes, mycorrhizae, biological control organisms, other culturable organisms, or the myriad of organisms in habitats occupied by *P. sulphurascens* that cannot be cultured. DNA-based identification of critical microbes within microbial communities in forest soils and endophyte communities of host roots would represent a critical first step to understanding the biotic interactions that occur within a well-characterized physical environment.

One approach is to characterize the entire microbiome within specific habitats occupied by *P. sulphurascens* during different stages in its life cycle. Mendes et al. (2011) used this approach to decipher the rhizosphere microbiome for sugar beet seedlings growing in soils suppressive and conducive, respectively, to the soil-borne pathogen *Rhizoctonia solani*. The suppressive soil was from a field cropped the previous year to sugar beets that was severely affected by Rhizoctonia root rot, confirming what has been observed for certain other root diseases of agricultural crops, which is that an outbreak of the disease in one crop can then result in the soil becoming microbiologically suppressive to that disease of that same crop grown in that same field the next year. The conducive soils came from the uncropped margin of the field cropped to sugar beets, and therefore should have been physically and chemically identical to the suppressive soil.

Metagenomic DNA was isolated and sequenced from the rhizosphere of sugar beet seedlings grown in, respectively, the original suppressive and conducive soils, suppressive soil

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pasteurized at 50° and 80° C, respectively, and conducive soil amended with 10% suppressive soil. The analysis revealed 33,346 operational taxonomic units of bacteria and archeae identified by a high-density 16S ribosomal DNA (rDNA) oligonucleotide microarray, referred to as a PhyloChip. No significant differences were found in the number of bacterial taxa among the different soil treatments, but rather, a unique cluster of bacterial taxa were found for each of the soil treatments. These results indicated that the relative abundance of several bacterial taxa was a better indicator of suppressiveness than the exclusive presence of specific bacterial taxa. Follow-on research narrowed the pool of candidate taxa responsible for disease suppression to the Pseudomonadaceae, Burkholderiaceae, Xanthomonadales, and Lactobacillaceae.

The ecological roles and impacts of forest root diseases are likely influenced by numerous diverse interacting factors. Continued studies are needed to better characterize the interacting roles of: 1) the hosts and pathogens at the species, stand, and individual tree levels; 2) the distribution, role, and influence of the associated biotic components of the forest ecosystems, including rhizosphere microorganisms, endophytes, mycorrhizal fungi, flora, and fauna in the understory and overstory; 3) the abiotic environment, including temperature, moisture, physical properties of soil (e.g., water-holding capacity, pH, organic content, nutrients, soil texture, soil depth, parent material, and other properties), latitude, elevation, slope, aspect, etc.; and 4) stand history, including fire (intensity and frequency), management (thinning, harvest, site preparation, regeneration, seed/seedling sources, etc.), and other biotic and abiotic disturbances on forest root diseases. Integrative approaches are needed that address multiple interacting factors that contribute to root disease and associated growth losses in forest ecosystems. The new tools of transcriptomics and metagenomics used to characterize the microbiome of specific and strategically selected environments or habitats of pathogens will make it possible to get a simultaneously more global yet more local view of the critical biological interaction(s) important in the life cycle of *P. sulphurascens* and the epidemiology of LRR.

Breeding for Genetically Based Resistance in Douglas-Fir to Laminated Root Rot

Given that both Douglas-fir and *P. sulphurascens* are indigenous to the forests of the Pacific Northwest, sources of natural disease resistance could have evolved. Countering this notion, the Committee speculated that even a 10,000-year coexistence would be too short for a significant degree of genetically based resistance to have become established by selection. Each rotation of Douglas-fir could take several centuries while thousands if not tens of thousands of generations over millions of years might be required for resistance to develop by natural variation and selection such as to establish phenotypes of Douglas-fir resistant to LRR at high frequency. There is very little opportunity for selective pressure to favor the increase of resistant phenotypes in forests because: 1) disease pressure normally

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only begins to occur in stands when all individuals, both resistant and susceptible, have become reproductive; 2) susceptible young trees may escape root disease during their first decades of regeneration so that resistant seedlings are not favored; and 3) resistant trees may not survive the outcomes of stand degradation from LRR, such as increasing Douglas-fir bark beetle attacks or fire. Susceptibility of mature resistant trees to bark beetle attacks would be particularly true for resistance of only intermediate effectiveness, in which trees carrying a low level of resistance and that have lost some water transport capacity may have sufficient stress under high beetle populations or drought to succumb to attack. The regular return of fire also resets ecological succession to allow a generation of resistant early seral species during which inoculum attenuates, thereby relaxing selection for LRR resistance when Douglas-fir reestablishes.

Nevertheless, there is evidence from research done in British Columbia of some potentially useful level of genetic variation in susceptibility/resistance of coastal Douglas-fir to *P. sulphurascens*. Beginning in 1992, the Sturrock team initiated a screening trial with 97 full-sib families provided by the B.C. Ministry of Forests and Range (BCMFR) coastal Douglas-fir Tree Improvement Program using two isolates of *P. sulphurascens* and found evidence of useful genetically based variation in susceptibility/resistance of coastal Douglas-fir to the fungus (Sturrock, 2005).

This discovery, and the identification and ranking of families with differential susceptibility to *P. sulphurascens*, was followed by the combined use of an in vitro inoculation technique (Sturrock et al., 2007) and histological and molecular techniques to identify 1) candidate pathogenicity-related (PR) genes and proteins involved in the defense response of wild-type and full-sib Douglas-fir to *P. sulphurascens* (Zamani et al., 2003, 2004; Sturrock et al., 2007; Islam et al., 2008, 2009, 2010, 2012), and 2) and candidate PR genes in *P. sulphurascens* (Williams et al., in preparation).

To understand the host side of the host-pathogen interaction, inoculation of laboratory-grown Douglas-fir seedlings with *P. sulphurascens* showed that infection in roots was initiated within two days of inoculation, but that Douglas-fir responds by activating many different defense-related proteins and genes, including a 10.6-kDa antimicrobial peptide, an endochitinase-like protein, a PR10 protein, a thaumatin-like protein (Robinson et al., 2000; Sturrock et al., 2007; Islam et al., 2009), Barwin-like PR2 genes (Islam et al., 2012), a disease resistant protein (Islam et al., 2008), and class IV chitinase (PR4) genes (Islam et al., 2010). Using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) on *P. sulphurascens*-infected Douglas-fir root samples, Islam et al., (2013) recently reported that infection significantly elevated expression of 12 diverse genes putatively encoding for defense-related proteins ranging from a defensin to a peroxidase to a "toll-interleukin-like receptor-nucleotide binding site domains-leucine rich repeat protein."

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On the pathogen side, investigation of pathogenicity-related genes in *P. sulphurascens* has thus far identified genes encoding the cell wall-loosening protein, expansin, and cell wall-degrading enzymes such as endogluconase, pectinesterase, and polysaccharide deacetylase. Other genes identified in *P. sulphurascens* and generally considered important to the virulence of phytopathogenic fungi include those coding for tetraspanins, hydrophobins, and metalloproteases (Williams et al., in preparation; Islam et al., 2013).

Breeding and testing has been ongoing with Douglas-fir in western North America for more than 50 years (Prudham, 2005) and could be expanded to include breeding for resistance to LRR, if phenotypes with some level of resistance or tolerance, or preferred activity in genes known to be involved in resistance, could be identified. Once useful variation is identified for a desired phenotype, as done in most advanced crop and livestock breeding programs today, molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSR) can be used to identify and select for the desired genotype(s), thereby precluding the need to grow out large segregating progenies of the species.

The in vitro inoculation technique developed by Sturrock et al. (2007) has enabled further elucidation of several aspects of the Douglas-fir/*P. sulphurascens* interaction at both the cellular and molecular levels. These include the discovery and first-time documentation of structures (e.g., haustoria, appressoria) involved in the infection process of *P. sulphurascens* and new knowledge on the occurrence and localization of host pathogenesis-related (PR) proteins in infected Douglas-fir tissues (Sturrock et al., 2007). The Sturrock team also has a unique collection of North American isolates of *P. sulphurascens* and *P. weirii* (Figure 4), possibly the largest such collection in the world as well as several transcriptomes created in 2011 from healthy, *P. sulphurascens*-challenged, and drought-stressed Douglas-fir seedlings.

In collaboration with Dr. Jörg Bohlmann's lab at University of British Columbia, the Sturrock team also investigated the potential for a new approach to protect Douglas-fir seedlings from *P. sulphurascens* infection by application or altered expression of the signal-molecule methyl jasmonate (MeJA) to initiate terpenoid production and the formation of traumatic resin ducts. Terpenoids of various structural types, including monoterpenes, sesquiterpenes, and diterpene resin acids, are critical components of the oleoresin-based defenses of many conifers. While resin ducts are a normal anatomical feature in conifer tissues and organs, formation of traumatic resin ducts in the developing secondary xylem is generally only observed after insect attack, fungal elicitation, or mechanical wounding (Hudgins et al., 2004; Stoffel, 2008). The results of the team's research with application of MeJA "provided the first description of the effects of MeJA applied to roots through the soil on the anatomy and terpene chemistry of a gymnosperm" (Huber et al., 2005b).

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It also identified and quantified the relative concentrations of 35 different terpenoids in MeJA-treated Douglas-fir seedlings and demonstrated that MeJA could induce traumatic resin duct formation in roots and stems of this species. In a related paper, Huber et al. (2005a) also characterized four terpene synthase cDNAs in Douglas-fir, thus providing an approach to understanding the molecular genetics and biochemistry of terpenoid resin defenses in this important species.

Genome Modification by Transcription Activator-Like Effectors (TALEs)

Of the many genome-modification technologies to emerge in the past 30 years, including transposon mutagenesis, *Agrobacterium*-mediated genetic transformation, and RNA interference (RNAi), possibly none are more widely applicable or represent greater simplicity than transcription activator-like effectors (TALEs) (Voytas & Joung, 2009; Pennisi, 2012b). TALEs were first discovered as a mechanism used by different species or pathotypes of plant pathogenic *Xanthomonas* to shut down the defenses of their hosts (Boch et al., 2009; Moscou & Bogdanove, 2009). These DNA-binding proteins have in common a variable number of tandem 34-amino acid repeats that are conserved except for two adjacent and highly variable amino acid residues at positions 12 and 13. It is these two amino acid residues that determine the DNA-binding specificity of TALEs. Specific di-amino acid residues within the repeat unit recognize and bind to individual nucleotides that make up the DNA molecule, i.e., thymine, adenine, cytosine, and quanosine, in the targeted genome—one di-amino acid residue to one nucleotide. The ability to vary di-amino acid residues within each tandem repeat of the TALE offers the means to modify almost any DNA sequence. While the pathogenic xanthomonads use a Type III injection system to introduce TALEs into their host nucleus, these DNA-binding proteins can also be artificially introduced into targeted cells. The addition of a nuclease to specific TALEs to produce TALENs (transcription activator-like effector nucleases) provides the additional means to cut or splice DNA at specific sites. Indeed, TALEs and TALENs are now becoming a commodity for the specific modification of genomes in crop plants, livestock, and human embryonic stem cells along with fundamental studies on model organisms (Bogdanove & Voytas, 2011; Pennisi, 2012b).

As more information becomes available on the transcriptome of the Douglas-fir/*P. sulphurascens* pathosystem, including expressed genes associated with host defense and pathogen virulence, it could be possible, through genome modification with TALEs or TALENs, to produce a clone of Douglas-fir with resistance and possibly even immunity to *P. sulphurascens*. Because of the modular protein architecture of TALEs, it is possible to construct artificial effectors for almost any specificity (Boch et al., 2009; Bogdanove & Voytas, 2011).

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Findings and Recommendations

Root diseases in managed western forests are a major contributor to the loss in timber productivity, revenues, and environmental benefits—negative impacts that will likely continue to increase, especially in the context of climate change. Because forest root diseases are the result of complex ecological interactions among the host tree, root pathogen, and diverse abiotic and biotic components of the root and wider ecosystem, it is essential to integrate diverse science disciplines and currently available and emerging research tools to understand and address the diverse interacting factors that contribute to forest root diseases, as illustrated in this report focused on the LRR.

The Committee's recommendations are listed below in two groups: 1) contemporary economical and proven management strategies and practices, some already practiced, that can be implemented or enhanced without delay; and 2) opportunities for research to better understand LRR of Douglas-fir and the myriad of interactions at the molecular and genetic levels.

Strategies and Policies for Management of Laminated Root Rot

The strategies and policies summarized below for management of LRR are based on what can be implemented without delay, with the goal to minimize or reduce risk to Trust assets while maximizing both timber production and ecosystems services in DNR-managed forests. The Committee recommends further, or at least anticipates, that implementation of these strategies and policies by the DNR will serve as an example for management of privately owned timber-producing forests in Washington and throughout the Pacific Northwest. Finally, these recommendations can and should serve as a foundation for greater understanding and better management of *Armillaria* root disease, and *Heterobasidion* root and butt rot, as well as LRR.

1. Forest pathologists should provide training opportunities, technical assistance, and consultation services for DNR foresters who deal with LRR.
2. Foresters should learn to identify LRR, maintain high awareness, and recognize the disease in forest communities. Several insects and other pathogens cause trees to produce symptoms similar to those caused by *P. sulphurascens*. Program managers should provide adequate training opportunities to DNR foresters. Experienced specialists should be made available when questions occur.
3. Foresters should conduct surveys to determine where LRR occurs within stands. An accurate understanding of the location of disease centers is necessary to make an appropriate treatment response and to determine whether treatments are working.

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4. Recording the location of infected trees or stumps after regeneration harvests will be key to enabling future responses, so foresters should consider adding a requirement to harvest contracts to collect LRR data or determine other means of accomplishing this task. All managers responsible for the unit should be made aware of the extent of the disease in the stand.
5. It is imperative that detailed records be kept on the location of the disease within stands and across the region, and representative isolates should be genetically characterized to ensure accurate identification. A standardized, robust system for capturing spatially explicit root disease observations and responses should be implemented. Adopt principles of precision agriculture, allowing this data-layer to be used in real-time overlays along with other stand and site characteristics. The pathogen can persist for decades in a stand and will likely persist through many generations of land managers.
6. Explore the option of accelerating harvest plans to capture the future mortality, actively cutting around disease pockets, creating non-host tree buffers and reforesting with non-host tree species, and forgo multiple partial-cut entries in stands with diffuse LRR to choose stand conversion to non-host species. Where available, use models that use best-current-knowledge of growth and yield dynamics for stands with root disease to schedule the most economically advantageous entry time for stand conversion. Any of these actions that starve the pathogen will limit its ability to infect in the future.
7. In sites known to be infested, favor tree species and promote stand conditions that will be less susceptible to *P. sulphurascens* when thinning and reforesting. Use resistant species that are suited to the site and will not initiate problems with different pathogens. Coordinate harvest layouts and extraction to protect valuable advanced reproduction species that occupy root-rot centers, if these are non-hosts.
8. Where work is cost effective and will not cause unacceptable damage to forest soils, consider removing infected trees along with their stumps and roots.
9. Be cautious about classifying entire stands if only portions are actually diseased, because treatments to control root rot can be expensive.
10. Be cautious to conduct thorough evaluations of mortality causal agents. Douglas-fir beetle mortality around LRR centers can often disguise the presence of root rot. Conversely, do not assume that all dead trees in a stand known to have LRR are infected or that LRR is the only root disease present. Many agents can kill trees, and some require different treatment regimens.

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11. Avoid thinning or pruning in and around infection centers because many trees in close proximity to infection centers, even those with green crowns, will likely have extensive root rot and are subject to windthrow when the stand is thinned. The investment in these practices is unlikely to be recovered.
12. Avoid planting infested, untreated sites with highly susceptible species. These individuals will provide a link for the pathogen to survive from one generation to the next.
13. Pine is considered safe for LRR centers, but unless one understands rust resistance and implements practices to reduce potential impacts of this disease, planting pine may not produce the desired benefit. If no disease treatment is applied, then managers should plan for a shorter rotation and reduce their expected timber yields from the unit. LRR, either directly through mortality or growth loss, or indirectly through windthrow, will limit stand yields.
14. Monitor treatments for success to determine what works and what does not work. Forest pathologists should assist in establishing and implementing rigorous monitoring protocols and developing technical assistance guidance information from the conclusions gleaned.
15. Managers should develop a system to acknowledge and reward foresters who detect and respond appropriately to reduce the impact of LRR on State Trust Lands over time.
16. Forest managers and researchers should coordinate to develop community- or ecosystem-based approaches to understand complex forest ecosystem interactions that contribute to LRR and develop approaches to reduce negative impacts of LRR.

Research Opportunities at the Molecular and Genetic Levels

1. Revisit and expand investigations of the population structure and phylogeography of *P. sulphurascens* and *P. weirii*, starting with the large culture collection already in existence in the lab of Rona Sturrock, with the intent to produce a more complete global picture of the genetic diversity and continental migration of this pathogen.
2. Conduct a comprehensive phylogenetic analysis of both *P. sulphurascens* and *P. weirii* from different hosts and at the global level to better understand the phylogenetic and evolutionary relationships between these two species.
3. Select/identify a unique DNA sequence, such as in the ITS region, to facilitate detection/diagnosis of *P. sulphurascens* using real-time PCR, and make this technology readily available to foresters in cases where confirmation of a diagnosis is needed.

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4. Use transcriptomics to identify genes uniquely expressed during successive stages in the Douglas-fir/*P. sulphurascens* interaction under controlled conditions, starting with prepenetration (ectotropic) growth of the pathogen in the infection court, followed by penetration of the bark, bridging the phloem and cambium, and initiation of decay in the sapwood. Similarly, identify the genes uniquely transcribed by the pathogen during its saprophytic existence as endophytic mycelia behind the zone lines formed by the fungus in dead wood.
5. Use metagenomics to characterize the microbiome of the rhizosphere of healthy (noninfected) and *P. sulphurascens*-infected Douglas-fir roots. Consider sampling roots from within and outside infection centers of a managed and natural stand of Douglas-fir. Consider a particular focus on root endophytes associated with healthy vs. diseased Douglas-fir trees.
6. Use metagenomics to investigate the comparative microbiomes, including endophytes, of roots of Douglas-fir seedlings established naturally from seed trees left after harvest vs. seedlings produced in a nursery for replanting. Collect environmental metagenomics data to determine environmental factors that are driving shifts in the soil microbiota that are associated with LRR.
7. Investigate the potential use of transcription activator-like effectors (TALEs) and TALEs modified to include nucleases (TALENs) as a means to modify specific DNA sequences in the genome of a Douglas-fir clone based on knowledge of key defense-response genes in the host and whether more-timely expression or over-expression, for example, might be sufficient to "confuse" the pathogen and result in useful genetically based resistance.
8. Complete genomic sequencing of *P. sulphurascens* along with the tools of bioinformatics to maximize interpretation and utility of the information revealed by the complete genomic sequence. The Department of Energy might be persuaded to take on this project based on the project's potential to reveal genes for novel enzymes with potential to digest lignocelluloses that would be useful in conversion of woody biomass to sugars for fermentation to biofuels, and as a means to increase the supply of woody biomass feedstock from conifers through better management of LRR. Catabolic enzymes of *P. sulphurascens* could also prove useful for bioremediation projects.

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Conclusions

Forest root diseases are complex ecological processes that comprise numerous interactions among the biotic and abiotic environments. These diseases historically played beneficial roles in forest ecosystems by eliminating maladapted trees, fostering decomposition and nutrient cycling, creating wildlife habitat, and promoting ecological succession. However, forest root diseases are also contributing to major losses in forest growth and hence revenue from timber sales, and are predicted to become even more damaging under climate change as forest trees become maladapted and hence more susceptible to root diseases

Among the full complex of root diseases of Washington forests, most notably Armillaria root disease caused by different species of *Armillaria*, *Heterobasidion* root and butt rot caused by *Heterobasidion* occidentale and *H. irregulare*, and laminated root rot (LRR) caused by *Phellinus sulphurascens*, the Committee concluded that LRR of Douglas-fir was best suited for further scientific research toward applied management options. While focused on LRR, it is expected that the information and conclusions reached in the course of this study can serve as a model for a similar study of one or both of the other two root diseases.

The most important reasons for selection of LRR are: 1) within North America, LRR is uniquely a disease of forests in the Pacific Northwest, compared with the wider distributions of the other root diseases; 2) LRR is a relatively simple pathosystem, being limited to conifers, compared with *Armillaria* and *Heterobasidion* root diseases, which occur on both hardwoods and soft woods; and 3) LRR is particularly destructive on Douglas-fir, a major source of revenue for the Washington Department of Natural Resources.

Economic, Environmental, and Ecological Impacts of Laminated Root Rot of Douglas-Fir

Laminated root rot of Douglas-fir is strongly influencing tree mortality, growth, and yield in Douglas-fir-dominated forests in the Pacific Northwest. Washington DNR lands are particularly at risk to LRR because the disease is most destructive on coastal Douglas-fir, which includes some of the most valuable DNR-managed timberland. Most estimates of losses due to this disease within individual stands were made 30 or more years ago and range from 5% to 15%.

As an example of the cost of 5% loss, the DNR sold 781.5 mBF (1,844,135 m^3) of Douglas-fir from trust lands in western Washington for a bid of nearly $207 million during the 24-month period from July 2009 to June 2011. Assuming 5% more timber in the sale had LRR not been present, the harvested yield should have been 820.575 mBF (1,936,341 m^3)

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and brought in $217.3 million in the same bid. In addition, another 5% timber would have produced enough additional lumber to build 2,442 more houses.

LRR along with other root diseases also predispose trees to greater damage by insects. In addition, root diseases along with insect damage are having an impact on ecosystem and environmental services provided by forests, contributing, for example, to evidence that forests are shifting from sinks to sources of carbon dioxide when forests are needed for more not less carbon sequestration. Tree death or reduced tree growth from root disease can also be altering nutrient cycling; potentially leaving nitrogen and water unused in the soil that would normally be extracted by tree growth but may subsequently move to other environments.

Past Research that has Shaped Modern Understanding of Laminated Root Rot

Phellinus sulphurascens is an endemic pathogen in Washington forests. It is considered to have co-existed with Douglas-fir and other conifers dating back thousands of years. The first record of *P. sulphurascens* on Douglas-fir dates back to 1929, but the importance of LRR as a problem for Douglas-fir was not recognized until 1940. This was about 30 years after the first plantations of Douglas-fir were established by the U.S. Forest Service as replacements for harvested old-growth trees. *Phellinus sulphurascens* has been shown to survive saprophytically for decades in the stumps of harvested trees, waiting for the root of a new host tree to grow to it rather than growing through soil to contact a new host.

The closely related *Phellinus weirii*, first described in 1914 on western redcedar in Idaho and the principal cause of LRR of the Cupressaceae family, may overlap geographically and to some extent can occur in the same hosts as *P. sulphurascens*. These two species are most reliably distinguished by polymerase chain reaction (PCR) using species-specific primers.

Expression of laminated root rot may start within 5 to 10 years of establishing a stand and continue to progress throughout the life of the stand. The disease typically spreads radially outward from an infection center. Susceptible hosts may be killed at any age. Root rot centers tend to be occupied by a single clone (genet) of the pathogen and, where infection centers meet and coalesce into larger centers, the clones tend to maintain their separate identities. Investigations of genetic diversity of the pathogen using restriction fragment-length polymorphisms (RFLP) indicates that infection centers are initiated from basidiospores, but that subsequent spread within centers is vegetative and not by basidiospores, although some evidence exists to the contrary.

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Contemporary Management Options and Recommendations

To minimize the risk to Trust assets, DNR should implement practices to keep LRR at endemic levels, document its presence, and reduce its impact. The Committee recommends training DNR foresters to identify LRR, locate LRR on the landscape, mark infected trees and stumps, genetically characterize representative samples of the pathogen for accurate identification, keep records, implement treatments and monitor their success, and avoid planting heavily infested sites with susceptible species like Douglas-fir. If no disease treatment is applied to infected stands, managers should reduce their expected timber yields from the unit. Managers should also develop a system to acknowledge and reward foresters who detect and respond appropriately to reduce the impact of LRR.

Remote sensing methods such as aerial photography and LiDAR are available for estimating the location and size of infection centers but are less reliable than ground-based inspection of stands. Of the ground-based methods used to estimate the area of infection centers, most make some allowance for the presence of the pathogen beyond trees with obvious symptoms, e.g., 30 feet (10 m).

Thinning or partial cutting around or in close proximity to infection centers can expose the remaining trees to windthrow because these trees, even those with green crowns, will likely have extensive root decay. On the other hand, the roots of trees uprooted due to windthrow, in contrast to intact stumps, can potentially reduce the inoculum potential of *P. sulphurascens* by its exposure during its saprophytic survival to an aerial environment to which it is less adapted and subject to drying out, compared with the more stable and favorable subterranean environment. A method of harvest that pushes trees over, similar to windthrow, would potentially leave less inoculum available for infection of the next stand. "Stumping," where stumps are removed before replanting, will also reduce the inoculum available for the next stand. Both methods share the same limitations of need for heavy equipment and impracticality on steeper slopes. Nevertheless, any practice that will lower the amount of inoculum on a site before replanting can help lower the loss from LRR.

The most effective practice for limiting the development of LRR in new stands is to plant nonsusceptible trees such as red alder or cottonwood, or less susceptible conifers such as western redcedar or western white pine. However, because it can take a minimum of two to three years to produce an alternative seedling crop with the correct seed source/genetics, management decisions should be moved forward to the preharvest phase to allow time for nursery staff to meet for regeneration needs.

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Opportunities Ripe for Research, Particularly Molecular Research, with Potential to Better Inform Management Options

The Committee identified a number of opportunities to use existing and emerging tools in molecular genetics and genomics to better understand the dynamic ecological interactions that contribute to forest root diseases and open new approaches to its management. These include:

- Investigate the population structure and phylogeography of *P. sulphurascens*, including the role of basidiospores;
- Improve on and make available to foresters the means to rapidly and accurately identify *P. sulphurascens* and *P. weirii* using PCR;
- Conduct a comprehensive phylogenetic analysis of both *P. sulphurascens* and *P. weirii* from different hosts and at the global level;
- Use transcriptomics to identify both host defense and virulence genes expressed during successive stages in the Douglas-fir/*P. sulphurascens* host/pathogen interaction;
- Continue the search for and characterize at the molecular level potentially useful genetically based resistance in populations of Douglas-fir;
- Examine the potential for development of resistant clones of Douglas-fir through genome modification using transcription activator-like effectors (TALEs);
- Use metagenomics to characterize the microbiome of the rhizosphere of healthy (noninfected) and *P. sulphurascens*-infected Douglas-fir roots;
- Compare the microbiomes of nursery seedlings with naturally established seedlings and with nursery seedlings after use for reforesting; and
- Develop a high-density map of the *P. sulphurascens* genome through sequencing.

The implementation of any one of the studies listed above represents a significant challenge in the Pacific Northwest, in part because the capacity of the forest research community to address diseases in forest ecosystems generally, and forest root diseases specifically, has diminished to well below critical levels over recent years. Particularly concerning is the complete absence of teaching of forest pathology due to retirements at the state's two research universities. This situation can only be alleviated by increasing the capacity of

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forest pathology research and teaching, and also by integrating forest pathology with other disciplines. In this regard, there is an urgent need to attract disciplines outside of the traditional field of forest pathology to get involved in this research. Forest root diseases present ideal and unlimited opportunities for ecological studies commensurate with the kinds of studies underway for other environments and ecosystems, including the northern tundra, oceans, deserts, rainforests, and the human microbiome among the many examples.

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Literature Cited

Agee, J. K. (1998). The landscape ecology of western Washington forest fire regimes [Special issue]. *Northwest Science, 72*, 24-34.

Anand, R. (2010). *Endophytic colonization and nitrogen fixation by* Paenibacillus polymyxa *in association with lodgepole pine and western red cedar* (Ph.D. thesis). The Faculty of Graduate Studies (Soil Science). University of British Columbia, Vancouver, BC.

Angilletta, M. J. (2006). Estimating and comparing thermal performance curves. *Journal of Thermal Biology, 31,* 541-545.

Angilletta, M. J., Huey, R. B., & Frazier, M. R. (2010). Thermodynamic effects on organismal performance: Is hotter better? *Physiological and Biochemical Zoology*, *83,* 197-206.

Angwin, P. A., & Hansen, E. M. (1993). Pairing tests to determine mating compatibility in *Phellinus weirii. Mycological Research*, *97,* 1469-1475.

Aoshima, K. (1953). Wood rotting *Poria* from Japan II. *Bulletin of the Government Forest Experiment Station*, *59,* 57–64.

Arnerup, J., Lind, M., Olson, A., Stenlid, J., & Elfstrand, M. (2011). The pathogenic white-rot fungus *Heterobasidion parviporum* triggers non-specific defence responses in the bark of Norway spruce. *Tree Physiology*, *31*(11), 1262-1272.

Arnold, R. (1981). Nothing can stop this pulling machine. *Western Conservation Journal*, *38,* 38-42.

Austin, M. P. (2002). Spatial prediction of species distribution: An interface between ecological theory and statistical modeling. *Ecological Modelling*, *157*(2-3), 101-118.

Bae, H., Hansen, E. M., & Strauss, S. H. (1994). Restriction fragment length polymorphisms demonstrate single origin of infection centers in *Phellinus weirii. Canadian Journal of Botany*, *72,* 440-447.

Banik, M. T., Paul, J. A., Burdsall, H. H. Jr., & Cook, M. E. (1993). Serological differentiation of two forms of *Phellinus weirii. Mycologia*, *85,* 605-611.

Barton, G. M. (1967). Differences in phenolic extracts from healthy Douglas-fir roots and those infected with *Poria weirii. Canadian Journal of Botany*, *45,* 1545-1552.

Bascompte, J. (2009). Disentangling the web of life. *Science*, *325,* 416-419.

Bascompte, J. (2010). Structure and dynamics of ecological networks. *Science*, *329*, 765-766.

Baucom, R. S. & de Roode, J. C. (2011), Ecological immunology and tolerance in plants and animals. *Functional Ecology*, *25,* 18–28. doi:10.1111/j.1365-2435.2010.01742.x

Beale, J. D. (1992). *Laminated root rot: Ecological relationships and stand productivity impacts in coastal Douglas-fir ecosystems of British Columbia* (Master's thesis). University of British Columbia, Vancouver, BC.

Beiler, K. J., Durall, D. M., Simard, S. W., Maxwell, S. A., & Kretzer, A. M. (2009). Architecture of the wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. *New Phytologist*, *185,* 543-553.

Copyright © Washington State Academy of Sciences. All rights reserved.

Bennington, C. C., Fetcher, N., Vavrek, M. C., Shaver, G. R., Cummings, K. J., & McGraw, J. B. (2012). Home site advantage in two long-lived arctic plant species: Results from two 30-year reciprocal transplant studies. *Journal of Ecology*, *100,* 841-851.

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17,* 478-486.

Bever, J. D., Dickie, I. A., Facelli, E., Facelli, J. M., Klironomos, J., Moora, M., & Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution*, *25,* 486-478.

Bier, J. E., & Buckland, D. C. (1947). Relation of research in forest pathology to the management of second growth forests. I: *Poria weirii* root rot, an important disease affecting immature stands of Douglas-fir. *British Columbia Lumberman, 31*(2), 49-51, 64, 66.

Bloomberg, W. J. (1981). Estimation and prediction of losses caused by *Phellinus weirii* in second-growth fir stands in coastal British Columbia. In *Proceedings of the 29th Western International Forest Disease Work Conference* (pp. 59-60). Vernon, BC.

Bloomberg, W. J. (1990). Effect of stand conditions on advance of *Phellinus weirii* in Douglas-fir plantations. *Phytopathology*, *80,* 553-559.

Bloomberg, W. J., & Beale, J. D. (1985). Relationship of ecosystem to *Phellinus weirii* root rot on southern Vancouver Island. In *Proceedings of the 33rd Western International Forest Disease Work Conference* (pp. 20-28). Olympia, WA.

Bloomberg, W. J., Cumberbirch, P. M., & Wallis, G. W. (1980). *A ground survey method for estimating loss caused by* Phellinus weirii *root rot. I: Development of survey design. Information Report BC-R-3.* Victoria, BC. Pacific Forest Research Centre, Canadian Forestry Service.

Bloomberg, W. J., & Reynolds, G. (1985). Growth loss and mortality in laminated root rot infection centers in second-growth Douglas-fir on Vancouver Island. *Forest Science, 31,* 497-508.

Bloomberg, W. J., & Reynolds, G. (1988). Equipment trials for uprooting root-rot-infected stumps. *Western Journal of Applied Forestry, 3*, 80-82.

Bloomberg, W. J., & Wallis, G. W. (1979). Comparison of indicator variables for estimating growth reduction associated with *Phellinus weirii* root rot in Douglas-fir plantations. *Canadian Journal of Forest Research 9*(1), 76-81.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., & Hahn, S. (2009). Breaking the code of DNA binding specificity of TAL-Type III effectors. *Science*, *326,* 1509-1512.

Bogdanove, A. J., & Voytas, D. F. (2011). TAL effectors: Customizable proteins for DNA targeting. *Science*, *333,* 1843-1846.

Borer, E. T., Antonovics, J., Kinkel, L. L., Hudson, P. J., Daszak, P., Ferrari, M. J., . . . Rizzo, D. M. (2011). Bridging taxonomic and disciplinary divides in infectious disease. *EcoHealth*, *8,* 261-267.

Boyles, J.G., Seebacher, F., Smit, B., & McKechnie, A.E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integrative and Comparitive Biology, 51*, 676-690.

Copyright © Washington State Academy of Sciences. All rights reserved.

British Columbia Ministry of Forests. (1995). *Root Disease Management Guidebook*. Forest Practices Code. BCMOF.

Browning, J. E., Littke, W. R., Greetham, J., Sheldahl, M., & Mah, Z. (2002). *Aerial remote sensing technology: Characteristics of snags, cavity nest use and plant species in mortality centers at Silver Lake and Yacolt, Longview Timberlands Region* (Weyerhaeuser Tech. Rep. 502-1100).

Bruehl, G. W. (1975). Systems and mechanisms of residue possession by pioneer fungal colonists. In G. W. Bruehl (Ed.), *Biology and Control of Soil-Borne Plant Pathogens* (pp. 77-83). Symposium conducted at meeting of American Phytopathological Society, St. Paul, MN.

Buchanan, T. S. (1948). *Poria weirii*: Its occurrence and behaviour on species other than cedars. *Northwest Science, 22*, 7-12.

Buckland, D. C. (1955). Damage appraisal in immature stands. In *Proceedings of the Third Western International Forest Diseases Work Conference* (pp. 27-29). Spokane, WA.

Buckland, D. C., Molnar, A. C., & Wallis, G. W. (1954). Yellow laminated root rot of Douglas fir. *Canadian Journal of Botany, 32*, 69-81.

Burns, R. M., & Honkala, B. H. (Tech. Coords.) 1990. *Silvics of North America: 1. Conifers; 2. Hardwoods* (Agriculture Handbook 654, Vol. 2). Washington, DC: U.S. Department of Agriculture, Forest Service.

Cheatham, M. R., Rouse, M. N., Esker, P. D., Ignacio, S., Pradel, W., Raymundo, R., & Garrett, K. A. (2009). Beyond yield: Plant disease in the context of ecosystem services. *Phytopathology, 99*, 1228-1236.

Childs, T. W. (1949, March). *Office report*. Portland, OR: U.S. Department of Agriculture Division of Forest Pathology.

Childs, T. W. (1955). *Synopsis of present information concerning Poria weirii root rot in Douglas-fir* (Res. Note PNW-RN-116). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Childs, T. W. (1963). *Poria weirii* root rot. *Phytopathology, 53*, 1124-1127.

Childs, T. W., & Shea, K. R. (1967). *Annual losses from disease in Pacific Northwest forests* (Res. Bull. PNW-RB-020). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Ciampi, M. B., Meyer, M. C., Costa, M. J. N., Zala, M., McDonald, B. A., & Ceresini, P. C. (2008). Genetic structure of populations of *Rhizoctonia solani* anastomosis group-1 IA from soybean in Brazil. *Phytopathology, 98*, 932-941.

Cleary, M., Sturrock, R., & Hodge, J. (2011). Southern interior forest region: Laminated root disease Stand Establishment Decision Aid. *BC Journal of Ecosystems and Management 12*(2), 17-20.

Cleary, M. R., Arhipova, N., Morrison, D. J., Thomsen, I. M., Sturrock, R. N., Vasaitis, R., . . . Stenlid, J. (2013). Stump removal to control root disease in Canada and Scandinavia: A synthesis of results from long-term trials. *Forest Ecology and Management, 290*, 5-14.

Copyright © Washington State Academy of Sciences. All rights reserved.

Cohen, W. B., Harmon, M. E., Wallin, D. O., & Fiorella, M. (1996). Two decades of carbon flux from forests of the Pacific Northwest: Estimates from a new modeling strategy. *BioScience, 46*, 836-844.

Cook, R. J. (1992). Wheat root health management and environmental concern. *Canadian Journal of Plant Pathology, 14*, 76-85.

Cordero, O. X., Wildschutte, H., Kirkup, B., Proehl, S., Ngo, L., Hussain, F., & Polz, M. F. (2012). Ecological populations of bacteria act as socially cohesive units of antibiotic production and resistance. *Science, 337*, 1228-1231.

Cruickshank, M. G., Morrison, D. J., & Punja, Z. K. (1997). Incidence of *Armillaria* species in precommercial thinning stumps and spread of *Armillaria ostoyae* to adjacent Douglas-fir trees. *Canadian Journal of Forest Research, 27*, 481-490.

Cruickshank, M. G., Morrison, D. J., & Lalumière, A. (2011). Site, plot, and individual tree yield reduction of interior Douglas-fir associated with non-lethal infection by *Armillaria* root disease in southern British Columbia. *Forest Ecology and Management, 261,* 297-307.

Cunningham, S. C., & Read, J. (2003). Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? *New Phytologist, 157*, 55-64.

Dai, Y.-C. (1995). Changbai wood-rotting fungi. 3. The genus *Phellinidium* (Basidiomycetes) and a new species, *P. aciferum*. *Annales Botanici Fennici, 32*, 63-73.

Dai, Y.-C. (2004). First report of laminated root rot on *Sabina przewalskii* caused by *Phellinus weirii sensu stricto* in China. *Plant Disease, 88*, 573.

Dai, Y.-C. (2010). Hymenochaetaceae (Basidiomycota) in China. *Fungal Diversity, 45*, 131-343.

Dai, Y.-C., & Qin, G. F. (1998). *Phellinidium sulphurascens*—A forest pathogen in China. *Fungal Science, 13*, 101-107.

Dickman, A. & Cook, S. (1989). Fire and fungus in a mountain hemlock forest. *Canadian Journal of Botany, 67*, 2005-2016.

Doğan, H. H., & Karadelev, M. (2009). *Phellinus sulphurascens* (Hymenochaetaceae, Basidiomycota): A very rare wood-decay fungus in Europe collected in Turkey. *Turkish Journal of Botany, 33*, 239-242.

Dray, S., Pélissier, R., Couteron, P., Fortin, M.-J., Legendre, P., Peres-Neto, P. R., . . . Wagner, H. H. (2012). Community ecology in the age of multivariate multiscale spatial analysis. *Ecological Monographs*, *82,* 257-275.

Durrall, D. M., Jones, M. D., & Lewis, K. J. (2005). Effects of forest management on fungal communities. In J. Dighton, J. F. White, & P. Oudermans (Eds.), *The fungal community, its organization and role in ecosystems* (pp. 833-856). London: CRC Press.

Elad, Y., Cytryn, E., Meller-Harel, Y., Lew, B., & Graber, E. R. (2011). The biochar effect: Plant resistance to biotic stresses. *Phytopathologia Mediterranea, 50*, 335-349.

Copyright © Washington State Academy of Sciences. All rights reserved.

Ellison, C. E., Hall, C., Kowbel, D., Welch, J., Brem, R. B., Glass, N. L., & Taylor, J. W. (2011). Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *PNAS: Proceedings of the National Academy of Sciences of the United States of America, 108*, 2831-2836.

Ezenwa, V. O., Gerardo, N. M., Inouye, D. W., Medina, M., & Xavier, J. B. (2012). Animal behavior and the microbiome. *Science, 338*, 198-199.

Feder, J. L., Egan, S. P., & Nosil, P. (2012). The genomics of speciation-with-gene-flow. *Trends in Genetics, 28*, 342-350.

File, A. L., Klironomos, J., Maherali, H., & Dudley, S. A. (2012). Plant kin recognition enhances abundance of symbiotic microbial partner. *PLoS ONE 7*(9) :e45648. doi:10.1371/journal.pone.0045648

Filip, G. M., & Schmitt, C. L. (1979). Susceptibility of native conifers to laminated root rot east of the Cascade Range in Oregon and Washington. *Forest Science, 25*, 261-265.

Fontaine, C. (2011). The ecological and evolutionary implications of merging different types of networks. *Ecology Letters, 14*, 1170-1181.

Frankel, S. J. (Tech. Coord.). (1998). *User's guide to the western root disease model, version 3.0* (Gen. Tech. Rep. PSWGTR-165). Berkeley, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Franklin, J. F., & Hemstrom, M. A. (1981). Aspects of succession in coniferous forests of the Pacific Northwest. In D. C. West, H. H. Shugart & D. B. Botkin (Eds.), *Forest Succession: Concepts and Application* (pp. 212-249). New York, NY: Springer-Verlag.

Franklin, J. F., Mitchell, R. J., & Palik, B. J. (2007). *Natural Disturbance and stand development principles for ecological forestry* (Gen. Tech. Rep. NRS-19). Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.

Fraser, R. G., Beale, J. D., & Nevill, R. J. (1995). Reduction of *Phellinus weirii* inoculum in Douglas-fir stumps by the fumigant Telone II-B. *Canadian Journal of Forest Research, 25*, 63-68.

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution, 27*, 489-496.

Gavin, D. G., Hallett, D. J., Hu, F. S., Lertzman, K. P., Prichard, S. J., Brown, K. . . . Peterson, D. L. (2007). Forest fire and climate change in western North America: Insights from sediment charcoal records. *Frontiers in Ecology and the Environment, 5*, 499-506.

Gedney, D. R. (1981). *Occurrence of laminated root rot on non-federal timberlands in northwest Oregon, 1976* (Res. Note PNW-RN-381). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Genung, M. A., Bailey, J. K., & Schweitzer, J. A. (2012). Welcome to the neighbourhood: Interspecific genotype by genotype interactions in Solidago influence above- and belowground biomass and associated communities. *Ecology Letters, 15*, 65-73.

Gilbertson, R. L. (1974). *Fungi that decay ponderosa pine*. Tucson: University of Arizona Press.

Copyright © Washington State Academy of Sciences. All rights reserved.

Gillette, W. D. (1975). *Biology of* Poria weirii*: Sexuality and effect on height growth of Douglas-fir* (Master's thesis). University of Washington, Seattle, WA.

Goheen, D. J., & Hansen, E. M. (1993). Effects of pathogens and bark beetles on forests. In T. B. Schowalter & G. M. Filip (Eds.), *Beetle-Pathogen Interactions in Conifer Forests* (pp. 175-196). Academic Press, London, and New York, NY.

Goheen, E. M., & Hansen, E. M. (1994). Tree vigor and susceptibility to infection by *Phellinus weirii*: Results of field inoculations. In M. Johansson & J. Stenlin (Eds.), *Proceedings of the Eighth International Conference on Root and Butt Rots* (pp. 45-51). Uppsala, Sweden.

Götzenberger, L., de Bello, F., Bråthen, K. A., Davison, J., Dubuis, A., Guisan, A., Zobel, M. (2012). Ecological assembly rules in plant communities—approaches, patterns and prospects. *Biological Reviews, 87*, 111-127.

Grivet, D., Sebastiani, F., Alía, R., Bataillon, T., Torre, S., Zabal-Aguirrel, . . . González-Martínez, S. G. (2011). Molecular footprints of local adaptation in two Mediterranean conifers. *Molecular Biology and Evolution, 28*, 101-116.

Guisan, A., & Zimmermann, N. E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling, 135*, 147-186.

Hadfield, J. S. (1985). *Laminated root rot: A guide for reducing and preventing losses in Oregon and Washington forests.* Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region.

Hadfield, J. S., Goheen, D. J., Filip, G. M., Schmidt, C. L., & Harvey, R. D. (1986). *Root diseases in Oregon and Washington conifers*. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region.

Hadfield, J. S., & Johnson, D. W. (1977). *Laminated root rot—A field guide for reducing and preventing losses in Oregon and Washington forests.* Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Region.

Hansen, E. M. (1976). Twenty-year survival of *Phellinus (Poria) weirii* in Douglas-fir stumps. *Canadian Journal of Forest Research, 6*, 123-128.

Hansen, E. M. (1979a). Nuclear condition and vegetative characteristics of homokaryotic and heterokaryotic isolates of *Phellinus weirii*. *Canadian Journal of Botany, 57*, 1579-1582.

Hansen, E. M. (1979b). Sexual and vegetative compatibility reactions in *Phellinus weirii*. *Canadian Journal of Botany, 57*, 1573-1578.

Hansen, E. M. (1979c). Survival of *Phellinus weirii* in Douglas-fir stumps after logging. *Canadian Journal of Forest Research, 9*, 484–488.

Hansen, E. M. (1986). Inoculation of Douglas-fir roots with isolates of *Phellinus weirii* on sites differing in root rot severity. *Canadian Journal of Forest Research, 16*, 619-623.

Hansen, E. M. (1995). Does *Fuscoporia heinrichii* cause laminated root rot in Douglas-fir? Pages In R. L. Mathiasen (Ed.), *Proceedings of the 43rd Western International Forest Disease Work Conference* (pp. 146-149), August 29 – September 1, 1995. Big Mountain Resort, Whitefish Montana.

Copyright © Washington State Academy of Sciences. All rights reserved.

Hansen, E. M., & Goheen, E. M. (2000). *Phellinus weirii* and other native root pathogens as determinants of forest structure and process in Western North America. *Annual Review of Phytopathology, 38*, 515–539.

Hare, J. D. (2012). How insect herbivores drive the evolution of plants. *Science, 338*, 50-51.

Heegaard, E. (2002). The outer border and central border for species-environmental relationships estimated by non-parametric generalised additive models. *Ecological Modelling, 157*, 131-139.

Heil, M., & Bostock, R. M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defenses. *Annals of Botany, 89*, 503-512.

Hennon, P. E. (1991). Diseases, insects, and animal damage of yellow cypress. In J. D. Lousier (Ed.), *Yellow cypress: Can we grow it? Can we sell it: Proceedings of a symposium,* March 26-28, 1990. Nanaimo, BC.

Hibbs, D. E., & Cromack, K. Jr. (1990). Actinorhizal plants in the Pacific Northwest. In C. R. Schwintzer & J. D. Tjepkema (Eds.), *The Biology of* Frankia *and Actinorizal Plants* (pp. 343-363). New York, NY: Academic Press.

Hood I. A., Redfern D. B., & Kile G. A. (1991). *Armillaria* in planted hosts. In: C. G. Shaw III & G. A. Kile (Eds.), *Armillaria* Root Disease., *Agricultural Handbook No. 691*, 122-49. Washington, D. C., U. S. Department of Agriculture.

Huber, D. P. W., Philippe, R. N., Godard, K.-A., Sturrock, R. N., & Bohlmann, J. (2005a). Characterization of four terpene synthase cDNAs from methyl jasmonate-induced Douglas-fir, *Pseudotsuga menziesii. Phytochemistry, 66*, 1427-1439.

Huber, D. P. W., Philippe, R. N., Madilao, L., Sturrock, R. N., & Bohlmann, J. (2005b). Changes in anatomy and terpene chemistry in roots of Douglas-fir seedlings following treatment with methyl jasmonate. *Tree Physiology, 25*, 1075-1083.

Hudgins, J. W., Christiansen, E., & Franceschi, V. R. (2004). Induction of anatomically based defense responses in stems of diverse conifers by methyl jasmonate: A phylogenetic perspective. *Tree Physiology, 24*, 251–264.

Hufford, K. M., & Mazer, S. J. (2003). Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution, 18*, 147-155.

Hunt, J., Bussie, L. F., Jennions, M. D., & Brooks, R. (2004). What is genetic quality? *Trends in Ecology & Evolution, 19*, 329-333.

Inglese, S. J., & Paul, N. D. (2006). Tolerance of *Senecio vulgaris* to infection and disease caused by native and alien rust fungi. *Phytopathology, 96*, 718-726.

Ishizuka, W., & Goto, S. (2011). Modeling intraspecific adaptation of *Abies sachalinensis* to local altitude and responses to global warming, based on a 36-year reciprocal transplant experiment. *Evolutionary Applications, 5*, 229-244. doi:10.1111/j.1752-4571.00216.x

Islam, M. A., Sturrock, R. N., & Ekramoddoullah, A. K. M. (2008). A proteomics approach to identify proteins differentially expressed in Douglas-fir seedlings infected by *Phellinus sulphurascens*. *Journal of Proteomics, 71*, 425-438.

Copyright © Washington State Academy of Sciences. All rights reserved.

Islam, M. A., Sturrock, R. N., & Ekramoddoullah, A. K. M. (2010). Identification, characterization, and expression analyses of class II and IV chitinase genes from Douglas-fir seedlings infected by *Phellinus sulphurascens*. *Phytopathology, 100*, 356-366.

Islam, M. A., Sturrock, R. N., & Ekramoddoullah, A. K. M. (2012a). Molecular cloning and gene transcription analyses of barwin-type PR-4 genes from *Phellinus sulphurascens*-infected Douglas-fir seedlings. *Forest Pathology, 42*, 279-288. doi:10.1111/j.1439-0329.2011.00752.

Islam, M. A., Sturrock, R. N., & Ekramoddoullah, A. K. M. (2013). Gene expression profiling of a compatible interaction between Douglas-fir and the root rot fungal pathogen *Phellinus sulphurascens*. *Phytopathology.* Submitted for publication.

Islam, M. A., Sturrock, R. N., Holmes, T. A., & Ekramoddoullah, A. K. M. (2009). Ultrastructural studies of *Phellinus sulphurascens* infection of Douglas-fir roots and immunolocalization of host pathogenesis-related proteins. *Mycological Research, 113*, 700-712.

Jeong, S.-C., & Myrold, D. D. (2001). Population size and diversity of *Frankia* in soils of *Ceanothus velutinus* and Douglas-fir stands. *Soil Biology & Biochemistry, 33*, 931-941.

Johnson, A. L. S., Wallis, G. W., & Foster, R. E. (1972). Impact of root rot and other diseases in young Douglas-fir plantations. *Forestry Chronicle, 48*, 316-319.

Johnson, D. W., & Wear, J. F. (1975). Detection of *Poria weirii* root rot centers in the Pacific Northwest with aerial photography. *Plant Disease Reporter, 59*, 77-81.

Kastner, W. W., Goheen, D. J., & Edmonds, R. L. (1994). Relationship between occurrence of laminated root rot and site characteristics in Douglas-fir forests of the northern Oregon Coast Range. *Western Journal of Applied Forestry, 9*, 14-l7.

Kause, A., & Odegard, J. (2012). The genetic analysis of tolerance to infections: A review. *Frontiers in Genetics, 3*, 262.

Kause, A., van Dalen, S., & Bovenhuis, H. (2012). Genetics of ascites resistance and tolerance in chicken: A random regression approach. *G3, 2*(5), 527-535.

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters, 7*, 1225-1241.

Keech, O., Carcaillet, C., & Nilsson, M. C. (2005). Adsorption of allelopathic compounds by wood-derived charcoal: The role of wood porosity. *Plant and Soil, 272*, 291-300.

Kile, G. A., McDonald, G. I., & Byler J. W. (1991). Ecology and disease in natural forests. In C. G. Shaw III & G. A. Kile (Eds.), *Armillaria* Root Disease., *Agricultural Handbook No. 691*, pp. 102-121). Washington, D. C., *US Department of Agriculture.*

Kinzig, A. P., Perrings, C., Chapin, F. S. III, Polasky, S., Smith, V. K., Tilman, D., & Turner, B. L. II. (2011). Paying for ecosystem services—Promise and peril. *Science, 334*, 603-604.

Kliejunas, J. T., Geils, B., Glaeser, J. M., Goheen, E. M., Hennon, P., Kim, M.-S., . . . Frankel, S. J. (2009). *Review of literature on climate change and forest diseases of western North America* (Gen. Tech. Rep. PSW-GTR-225). Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Copyright © Washington State Academy of Sciences. All rights reserved.

Kolton, M., Harel, Y. M., Pasternak, Z., Graber, E. R., Elad, Y., & Cytryn, E. (2011). Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Applied and Environmental Microbiology, 77*, 4924-4930.

Kotiranta, H. K., Mukhin, V. A., Ushakova, N., & Dai, Y.-C. (2005). Polypore (Aphyllophorales, Basidiomycetes) studies in Russia. 1. South Ural. *Annales Botanici Fennici, 42*, 427-451.

Kotlaba, F., & Pouzar, Z. (1970). Revision of the original material of *Phellinus sulphurascens* Pil., *Xanthochrous glomeratus* ssp. *heinrichii* and *Polyporus rheades* Pers. (Hymenochaetaceae). *Česká Mykologie, 24*, 146–152.

Laikre, L., Schwartz, M. K., Waples, R. S., N., & the GEM Working Group. (2010) Compromising genetic diversity in the wild: Unmonitored large-scale release of plants and animals. T*rends in Ecology & Evolution, 25*, 520-529.

Lane, B. B., & Goheen, D. J. (1979). Incidence of root disease in bark beetle-infested eastern Oregon and Washington true firs. P*lant Disease Reporter, 63*, 262-266.

Larsen, M. J., & Cobb-Poulle, L. A. (1990). *Phellinus* (Hymenochaetaceae). A survey of the world taxa. *Synopsis Fungorum, 3*, 1–206.

Larsen, M. J., Lombard, F. F., & Clark, J. W. (1994). *Phellinus sulphurascens* and the closely related *P. weirii* in North America. *Mycologia, 86*, 121–130.

Lawson, T. T. (1979). *Some silvicultural impacts of* Phellinus weirii *on a managed forest in the interior Coast Range of Oregon* (Master's thesis). Oregon State University, Corvallis, OR.

Lawson, T. T., Berg, A. L., & Hansen, E. M. (1983). *Damage from laminated root rot at the Black Rock Forest Management Research Area in western Oregon* (Res. Note 75). Corvallis, OR: Oregon State University, Forest Research Laboratory.

Leal, I., Bergeron, M. J., Hamelin, R. C., Foord, B., Pellow, K. W., Ross, G., & Sturrock, R. N. (2012). *Genetic characterization of* Phellinus sulphurascens *in Western North America and Eurasia*. Manuscript in preparation.

Leckie, D. G., Jay, C., Gougeon, F. A., Sturrock, R. N., & Paradine, D. (2004). Detection and assessment of trees with *Phellinus weirii* (laminated root rot) using high resolution multi-spectral imagery. *International Journal of Remote Sensing, 25,* 793-818.

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota – A review. Soil *Biology and Biochemistry, 43*, 1812-1836.

Leimu, R., & Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PLoS ONE 3*(12): e4010. doi:10.1371/journal.pone.0004010

Lewinsohn, T. M., & Cagnola, L. (2012). Keystones in a tangled bank. *Science, 335*, 1449-1451.

Li, C. Y. (1979). Light and temperature induced sporocarp formation of *Phellinus weirii*. *Canadian Journal of Forest Research, 9*, 535-538.

Lim, Y. W., Sturrock, R. N., Leal, I., Pell, K., Yamaguchi, T., & Breuil, C. (2008). Distinguishing homokaryons and heterokaryons in *Phellinus sulphurascens* using pairing tests and ITS polymorphisms. *Antonie van Leeuwenhoek International Journal of General and Molecular Biology, 93*, 99-110.

Copyright © Washington State Academy of Sciences. All rights reserved.

Lim, Y. W., Yeung, Y. C. A., Sturrock, R. N., Leal, I., & Breuil, C. (2005). Differentiating the two closely related species, *Phellinus weirii* and *P. sulphurascens*. *Forest Pathology, 35*, 305-314.

Lindemayer, D. B, Laurence, W. F., & Franklin, J. F. (2012). Global decline in large old trees. *Science, 338*, 1305-1306.

Lowry, D. B. (2012a). Local adaptation in The model plant. *New Phytologist, 194*, 888-890.

Lowry, D. B. (2012b). Ecotypes and the controversy over stages in the formation of new species. *Biological Journal of the Linnean Society, 106*, 241-257.

Magurran, A. E., & McGill, B. J. (Eds.). (2011). Biological Diversity: Frontiers in measurement and assessment. Oxford: Oxford University Press.

Maurano, M. T., Humbert, R., Rynes, E., Thurman, R. E., Haugen, E., Wang, H., . . . Stamatoyannopoulos, J. A. (2012). Systematic localization of common disease-associated variation in regulatory DNA. *Science, 337*, 1190-1195.

May, R. M. (2006). Network structure and the biology of populations. *Trends in Ecology & Evolution, 21*, 394-399.

Mazzola, M., & Manici, L. M. (2012). Apple replant disease: Role of microbial ecology in cause and control. *Annual Review of Phytopathology, 50*, 45-65.

McCauley, K. J., & Cook, S. A. (1980). *Phellinus weirii* infestation of two mountain hemlock forests in the Oregon Cascades. *Forest Science, 26*, 23-29.

McDonald, G. I., Harvey, A. E., & Tonn, J. R. (2000). Fire, competition, and forest pests: Landscape treatment to sustain ecosystem function. In L. F. Neunschwander & K. C. Ryan (Tech. Eds.), *Proceedings from the Joint Fire Science Conference and Workshop: Crossing the millennium: Integrating spatial technologies and ecological principles for a new age in fire management* (pp. 195-211). Volume 2. University of Idaho and the International Association of Wildland Fire, Moscow, ID.

McDonald, G. I., Zambino, P., & Klopfenstein, N. B. (2005). Naturalization of host-dependent microbes after introduction into terrestrial ecosystems. In J.E. Lundquist, & R.C. Hamelin (Eds). *Forest pathology: from genes to landscapes* (pp. 41-57). The American Phytopathological Society, St. Paul, MN.

McKinley, D. C., Ryan, M. G., Birdsey, R. A., Giardina, C. P., Harmon, M. E., Heath, L. S., & Skog, K. E. (2011). A synthesis of current knowledge on forests and carbon storage in the United States. *Ecological Applications, 21*, 1902-1924.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., & Raaijmakers, J. M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science, 332*, 1097-1100.

Montesinos-Navarro, A., Segarra-Moragues, J. G., Valiente-Banuet, A., & Verdú, M. (2012). The network structure of plant-arbuscular mycorrhizal fungi. *New Phytologist, 194*, 536-547.

Morrison, D. J., Merler, H., & Norris, D. (1992). *Detection, recognition and management of* Armillaria *and* Phellinus *root diseases in the southern interior of British Columbia* (FRDA Rep. 179). Victoria, BC: Forestry Canada, Pacific Forestry Centre, co-published by BC Ministry of Forests.

Copyright © Washington State Academy of Sciences. All rights reserved.

Morrison, D. J., Wallis, G. W., & Weir, L. C. (1988). *Control of* Armillaria *and* Phellinus *root diseases: 20-year results from the Skimikin stump removal experiment* (Inf. Rep. BC-X-302). Victoria, BC: Canadian Forestry Service, Pacific Forestry Centre.

Moscou, M. J., & Bogdanove, A. J. (2009). A simple cipher governs DNA recognition by TAL effectors. *Science, 326*, 1501.

Moslonka-Lefebvre, M., Finley, A., Dorigatti, I., Dehnen-Schmutz, K., Harwood, T., Jeger, M. J., Xu X., . . . Pautasso, M. (2011). Networks in plant epidemiology.

Mounce, I., Bier, J. E., & Nobles, M. K. (1940). A root-rot of Douglas-fir caused by *Poria weirii*. *Canadian Journal of Research, 18*, 522–533.

Mouquet, N., Devictor, V., Meynard, C. N., Munoz, F., Bersier, L.-F., Chave, J., & Thuiller, W. (2012). Ecophylogenetics: Advances and perspectives. *Biological Reviews, 87*, 769-785.

Murrill, W. A. (1914). An enemy of the western red cedar. *Mycologia, 6*, 93–94.

National Research Council (U.S.), National Research Council (U.S.), & National Research Council (U.S.). (2009). *A New Biology for the 21st Century.* Washington, DC: National Academies Press.

Nelson, E. E. (1971). *Invasion of freshly cut Douglas-fir stumps by* Poria weirii (Res. Note PNW-144). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Nelson, E. E. (1973). *Observations on the formation of zone lines in wood by* Poria weirii (Res. Note PNW-210). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Nelson, E. E. (1976). Colonization of wood disks initiated by basidiospores of *Phellinus weirii* (laminated root rot). *Forest Science, 22,* 407-411.

Nelson, E. E. (1980). *Laminated root rot damage in a young Douglas-fir stand* (Res. Note PNW-359). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station.

Nelson, E. E., & Fay, H. A. (1975). Effect of temperature on growth and survival of high- and low elevation isolates of *Phellinus* (*Poria weirii*). *Northwest Science, 49*, 119-121.

Nelson, E. E., & Hartman, T. (1975). Estimating spread of *Poria weirii* in a high-elevation, mixed conifer stand. *Journal of Forestry, 73*, 141-142.

Nelson, E. E., Martin, N. E., & Williams, R. E. (1981). *Laminated root rot of western conifers* (Forest Insect & Disease leaflet 159). Washington, DC: U.S. Department of Agriculture, Forest Service.

Nelson, E. E., & Sturrock, R. N. (1993). Susceptibility of western conifers to laminated root rot (*Phellinus weirii*) in Oregon and British Columbia field tests. *Western Journal of Applied Forestry, 8*, 67-70.

Newman, M. E. J. (2012). Communities, modules and large-scale structure in networks. *Nature Physics, 8*, 25-31.

Copyright © Washington State Academy of Sciences. All rights reserved.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., . . . van Kleunen, M. (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science, 15*, 684-692.

Nosil, P., & Feder, J.L. (2012). Genomic divergence during speciation: Causes and consequences. *Philosophical Transactions of the Royal Society B*, *367*, 332-342.

Ohmann, J. L. (2002). Snag dynamics in western Oregon and Washington. *DecAID - Decayed Wood Advisor Digital Report.* U. S. Department of Agriculture Forest Service and U. S. Fish and Wildlife Service. http://www.fs.fed.us/r6/nr/wildlife/decaid/pages/Snag-Dynamics.html

Oren, R., Thies, W. G., & Waring, R. H. (1985). Tree vigor and stand growth of Douglas-fir as influenced by laminated root rot. *Canadian Journal of Forest Research, 15*, 985-988.

Ostfeld, R. S., Keesing, F., & Eviner, V.T. (Eds.). (2008). *Infectious disease ecology: Effects of ecosystems on disease and of disease on ecosystems.* Princeton, NJ: Princeton University Press.

Ouborg, N. J., Pertoldi, C., Loeschcke, V., Bijlsma, R. K., & Hedrick, P. W. (2010). Conservation genetics in transition to conservation genomics. *Trends in Genetics, 26*, 177-187.

Parmasto, E., & Parmasto, I. (1979). The xanthochroic reaction in Aphyllophorales. *Mycotaxon, 8*, 201–232.

Pellow, K. W., & Sturrock, R. N. (2010). Induced fruiting of *Phellinus sulphurascens*. *Canadian Journal of Plant Pathology, 33*, 289.

Pennisi, E. (2012a). ENCODE project writes eulogy for junk DNA. *Science, 337*, 1159-1161.

Pennisi, E. (2012b). The tale of the TALEs. *Science, 338*, 1408-1411.

Petrova, R. D. (2012). New scientific approaches to cancer treatment: Can medicinal mushrooms defeat the curse of the century? *International Journal of Medicinal Mushrooms, 14*, 1-20.

Pilát, A. (1936). *Phellinus sulphurascens* Pilát. *Bulletin de la Société Mycologique de France 51(3-4)*, 372 (MB#354993).

Post, W. M., Izaurrabde, R. C., West, T. O., Liebig, M. A, & King, A. W. (2012). Management opportunities for enhancing terrestrial carbon dioxide sinks. *Frontiers in Ecology and Environment, 10,* 554-561.

Proulx, S. R., Promislow, D. E. L., & Phillips, P. C. (2005). Network thinking in ecology and evolution. *Trends in Ecology & Evolution, 20,* 345-353.

Prudham, W. Scott. (2005). *Knock on Wood: Nature as Commodity in Douglas-Fir Country.* Florence, KY: Psychology Press.

Reynolds, K. M, & Bloomberg, W. J. (1982). Estimating probability of inter-tree root contact in second-growth Douglas-fir. *Canadian Journal of Forest Research, 12*, 493-498.

Rishbeth, J. (1963). Stump protection against *Fomes annosus*: III. Inoculation with *Peniophora gigantea. Annals of Applied Biology, 52*, 63-77.

Copyright © Washington State Academy of Sciences. All rights reserved.

Robinson, R. M., Sturrock, R. N., Davidson, J. J., Ekramoddoullah, A. K. M., & Morrison, D. J. (2000). Detection of a chitinase-like protein in the roots of Douglas-fir trees infected with *Armillaria ostoyae* and *Phellinus weirii*. *Tree Physiology, 20,* 493-502.

Ross-Davis, A. L., Stewart, J. E., Hanna, J. W., Kim, M.-S., Knaus, B. J., Cronn, R., . . . Klopfenstein, N. B. (2013). Transcriptome of an *Armillaria* root disease pathogen reveals candidate genes involved in host substrate utilization at the host-pathogen interface. *Forest Pathology, 43*. In press, DOI: 10.1111/efp.12056.

Roux, F., Gao, L., & Bergelson, J. (2010). Impact of initial pathogen density on resistance and tolerance in a polymorphic disease resistance gene system in *Arabidopsis thaliana*. *Genetics 185*, 284-291.

Russell, K., R. Johnsey, & Edmonds, R. L. (1986). Disease and insect management. In C. D. Oliver, D. P. Hanley, & J. A. Johnson (Eds.), *Douglas fir. Stand management for the future* (pp. 189–207). Institute of Forest Products, College of Forest Resources, University of Washington, Contribution 55, Seattle, WA.

Ryan, C. A., & Jagendorf, A. (1995). Self defense by plants. *Proceedings of the National Academy of Science of the United States of America, 92*, 4075. doi: 10.2307/2367271

Ryvarden, L., & Gilbertson, R. L. (1994). European polypores 2. Meripilus-Tyromyces. *Synopsis Fungorum, 6*, 389-743.

Savolainen, O., Pyhajarvi, T., & Knurr, T. (2007) Gene flow and local adaptation in trees. *Annual Review of Ecology, Evolution, and Systematics, 38*, 595-619.

Schnitzer, S.A., Klironomos, J. N., HilleRisLambers, J., Kinkel, L. L., Reich, P. B., Xiao, K., . . . Scheffer, M. (2011). Soil microbes drive the classic plant diversity-productivity pattern. *Ecology, 92*, 296-303.

Sessitsch, A., Hardoim, P., Döring, J., Weilharter, A., Krause, A., Woyke, T., . . . Reinhold-Hurek, B. (2012). Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Molecular Plant-Microbe Interactions, 25*, 28-36.

Simard, S. W., Beiler, K. J., Bingham, M. A., Deslippe, J. R., Philip, L. J., & Teste, F. P. (2012). Mycorrhizal networks: Mechanisms, ecology and modelling. *Fungal Biology Reviews, 26*, 39-60.

Slavov, G. T., DiFazio, S. P., Martin, J., Schackwitz, W., Muchero, W., Rodgers-Melnick, E., & Tuskan, G. A. (2012). Genome resequencing reveals multiscale geographic structure and extensive linkage disequilibrium in the forest tree *Populas trichocarpa*. *New Phytologist, 196*, 713-725.

Smith, D. S., Schweitzer, J. A., Turk, P., Bailey, J. K., Hart, S. C., Shuster, S. M., & Whitham, T. G. (2012). Soil-mediated local adaption alters seedling survival and performance. *Plant and Soil, 352*, 243-251.

Stapley, J., Reger, J., Feulner, P. G. D., Smadja, C., Galindo, J., Ekblom, R., . . . Slate, J. (2010). Adaption genomics: The next generation. *Trends in Ecology & Evolution, 25*, 705-712.

Stewart, F. J., Sharma, A. K., Bryant, J. A., Eppley, J. M., & Delong, E. F. (2010). Community transcriptomics reveals universal patterns of protein sequence conservation in natural microbial communities. *Genome Biology, 12*, R26.

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Stinchcombe, J. R., Function-valued Traits Working Group, & Kirkpatrick. M. (2012). Genetics and evolution of function-valued traits: Understanding environmentally responsive phenotypes. *Trends in Ecology and Evolution, 27*, 637-647.

Stoffel, M. (2008). Dating past geomorphic processes with tangential rows of traumatic resin ducts. *Dendrochronologia, 26*, 53-60.

Sturrock, R. (2005). Investigations of Douglas-fir resistance to *Phellinus weirii. TICtalk, a publication of the Forest Genetics Council of British Columbia, 6*(1), 3-7. http://www.fgcouncil.bc.ca/tictalk-jun05-web.pdf

Sturrock, R. N. (2000). *Management of root diseases by stumping and push-falling* (Technology Transfer Note 16). Victoria, BC: Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.

Sturrock, R. N. (2012). Stumping for control of *Phellinus, Tomentosus and Annosus* root diseases: A synthesis of results from some North American trials. In S. Zeglen (Ed.), *Proceedings of the 59th Annual Western International Forest Disease Work Conference* (pp. 49-52). October 10-14, 2011. Leavenworth, WA.

Sturrock, R., & Garbutt, R. (1994). *Laminated root rot of Douglas-fir* (Canada and British Columbia Forest Pest Leaflet 3). Victoria, BC: Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.

Sturrock, R. N, Frankel, S. J., Brown, A. V., Hennon, P. E., Kliejunas, J. T., Lewis, K. J., . . . Woods, A. J. (2011). Climate change and forest diseases. *Plant Pathology, 60*, 133-149.

Sturrock, R. N., Islam, M. A., & Ekramoddoullah, A. K. M. (2007). Host-pathogen interactions in Douglas-fir seedlings infected by *Phellinus sulphurascens. Phytopathology, 97*, 1406-1414.

Sturrock, R. N., Pellow, K. W., & Hennon, P. E. (2010). *Phellinus weirii* and other fungi causing decay in western redcedar and yellow-cedar. In C. A. Harrington (Tech. coord.), *A tale of two cedars: International symposium on western redcedar and yellow-cedar* (Gen. Tech. Rep. PNW-GTR-828) (pp. 47-52). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Sturrock, R. N., Phillips, E. J., & Fraser, R. G. (1994). *A trial of push-falling to reduce Phellinus weirii infection of coastal Douglas-fir* (Forest Resource Development Agreement Report 217). Victoria, BC: Forestry Canada, B.C. Ministry of Forests, Canada-British Columbia Partnership Agreement on Forest Resource Development: FRDA II.

Sturrock, R. N., & Reynolds, G. (1998). A new technique for inoculation of conifer seedling roots with the laminated root rot pathogen *Phellinus weirii*. *Canadian Journal of Plant Pathology, 20*, 324-330.

Sun, H., Paulin, L., Alatalo, E., & Asiegbu, F. O. (2011). Response of living tissues of *Pinus sylvestris* to the saprotrophic biocontrol fungus *Phlebiopsis gigantea*. *Tree Physiology, 31*, 438-451.

Swanson, M. (2006). *An Ecological History of the Charles L. Pack Experimental Forest, Eatonville, WA: Natural History, Landscape Ecology, and Forest Management.* Special Report, Center for Sustainable Forestry at Pack Forest, College of Forest Resources, University of Washington, Seattle, WA.

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Tappeiner, J. C., Huffman, D. W., Marshall, D., Spies, T. A., & Bailey, J. D. (1997). Density, ages, and growth rates in old-growth and young-growth forests in coastal Oregon. *Canadian Journal of Forest Research, 27*, 636-648.

Thies, W. G. (1982). Growth loss as a measure of stress in second-growth Douglas-fir infected by *Phellinus weirii. In Proceedings of the 30th Western International Forest Disease Work Conference* (pp. 121-136). Fallen Leaf Lake, CA.

Thies, W. G. (1983). Determination of growth reduction in Douglas-fir infected by *Phellinus weirii*. *Forest Science, 29*, 305-315.

Thies, W. G., & Nelson, E. E. (1982). Control of *Phellinus weirii* in Douglas-fir stumps by the fumigants chloropicrin, allyl alcohol, Vapam, or Vorlex. *Canadian Journal of Forest Research, 12*, 528-532.

Thies, W. G., & Nelson, E. E. (1987). Reduction of *Phellinus weirii* inoculum in Douglas-fir stumps by the fumigants chloropicrin, Vorlex, or methylisothiocyanate. *Forest Science, 33*, 316-329.

Thies, W. G., & Nelson, E. E. (1988). Bulldozing stumps and nitrogen fertilization affect growth of Douglas-fir seedlings. *Canadian Journal of Forest Research, 18*, 801-804.

Thies, W. G., & Nelson, E. E. (1994). Therapeutic treatment of mature, living Douglas-fir with chloropicrin, methylisothiocyanate, or Vorlex reduces root colonization by the pathogen *Phellinus weirii. In Proceedings of the 8th International Conference on Root and Butt Rots* (pp. 736-741). Uppsala, Sweden.

Thies, W. G., & Nelson, E. E. (1997). Laminated root rot: New consideration for surveys. *Western Journal of Applied Forestry, 12*, 49-51.

Thies, W. G., & Sturrock, R. N. (1995). *Laminated root rot in western North America* (Gen. Tech. Rep. PNW-GTR-349). Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. In cooperation with: Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.

Thies, W. G., & Westlind, D. J. (2005). Stump removal and fertilization of five *Phellinus-weirii*-infested stands in Washington and Oregon affect mortality and growth of planted Douglas-fir 25 years after treatment. *Forest Ecology and Management, 219*, 242-258.

Thomas, M. B., & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution, 18*, 345-350.

Thomson, A. J., Goodenough, D. G., Barclay, H. J., Lee, Y. J., & Sturrock, R. N. (1996). Effects of laminated root rot (*Phellinus weirii*) on Douglas-fir foliar chemistry. *Canadian Journal of Forest Research, 26*, 1440-1445.

Thor, M. (2003). Operational stump treatment against *Heterobasidion annosum* in European forestry—current situation. In G. Laflamme, J. A. Bérubé, & G. Bussières (Eds.), *Proceedings of the Tenth International Conference on Root and Butt Rot*. September 16-22, 2003. Quebec City, Canada.

Tkacz, B. M., & Hansen, E. M. (1982). Damage by laminated root rot in two succeeding stands of Douglas-fir. *Journal of Forestry, 80*, 788-791.

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Trappe, J. M., Li, C. Y., Lu, K. C., & Bollen, W. B. (1975). Differential Response of *Poria weirii* to phenolic acids from Douglas-fir and red alder roots. *Forest Science, 19*, 191-196.

Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends in Ecology & Evolution, 23*, 432-438.

U.S. Forest Service. (1958). *Timber resources for America's future* (Forest Resource Rep. 14). Washington, DC: U.S. Forest Service, U.S. Department of Agriculture.

U.S. Forest Service. (2012). *Forest Vegetation Simulator (FVS)*. Retrieved from the U.S. Forest Service website: http:// http://www.fs.fed.us/fmsc/fvs/

van der Kamp, B. J. (1991). Pathogens as agents of diversity in forested landscapes. The *Forestry Chronicle, 67*, 353-354.

van Loon, L. C., Bakker, P. A. H. M., & Pieterse, C. M. J. (1998). Systemic resistance induced by rhizobacteria. *Annual Review of Phytopathology, 36*,453-483.

van Veelen, M., Garcia, J., Rand, D. G., & Nowak, M. A. (2012). Direct reciprocity in structured populations. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 9929-9934

Vellend, M. (2010). Conceptual synthesis in community ecology. *The Quarterly Review of Biology, 85*, 183-206.

Voytas, D. F., & Joung, K. J. (2009). DNA binding made easy. *Science, 326*, 1491-1492.

Wagner, T., & Fischer, M. (2002). Proceedings towards a natural classification of the worldwide taxa *Phellinus s.l.* and *Inonotus s.l.*, and phylogenetic relationships of allied genera. *Mycologia, 94*, 998–1016.

Wallis, G. W. (1959). *Poria* root rot. *In Proceedings of the 7th Western International Forest Disease Work Conference* (pp. 76-81). Pullman, WA.

Wallis, G. W. (1967). *Poria weirii* root rot. In A. G. Davidson & R. M. Prentice (Eds.), *Important forest insects and diseases of mutual concern to Canada, the United States and Mexico* (Canada Department of Forestry and Rural Development Publication 1180) (pp. 205-206).

Wallis, G. W. (1976a). Growth characteristics of *Phellinus (Poria) weirii* in soil and on root and other surfaces. *Canadian Journal of Forest Research, 6*, 229-232.

Wallis, G. W. (1976b). Phellinus (Poria) weirii *root rot: Detection and management proposals in Douglas-fir stands* (Forestry Tech. Rep. 12). Victoria, BC: Canadian Forestry Service, Pacific Forest Research Center.

Wallis, G. W., & Bloomberg, W. J. (1981). Estimating the total extent of *Phellinus weirii* root rot centers using above- and below-ground disease indicators. *Canadian Journal of Forest Research, 11,* 827-830.

Wallis, G. W., & Lee, Y. J. (1984). Detection of root disease in coastal Douglas-fir stands using large scale 70-mm aerial photography. *Canadian Journal of Forest Research, 14,* 523-527.

Wallis, G. W., & Reynolds, G. (1965). The initiation and spread of *Poria weirii* root rot of Douglas-fir. *Canadian Journal of Botany, 43*, 1-9.

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Washington State Blue Ribbon Panel on Ocean Acidification. (2012). *Ocean acidification: From knowledge to action, Washington state's strategic response.* H. Adelsman & L. Whitely Binder (Eds.). Olympia, WA: Washington Department of Ecology, Olympia, WA.

Weber, M. G., & Agrawal, A. A. (2012). Phylogeny, ecology, and the coupling of comparative and experimental approaches. *Trends in Ecology & Evolution, 27*, 394-403.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., . . . Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nature Reviews Genetics, 7*, 510-523.

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science, 320*, 492-495.

Whitham, T. G., Gehring, C. A., Lamit, L. J., Wojtowicz, T., Evans, L. M., Keith, A. R., & Smith, D. S. (2012). Community specificity: Life and afterlife effects of genes. *Trends in Plant Science, 17*, 271-281.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M., . . . Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology, 84*, 559-573.

Williams, H. L, Sturrock, R. N., Islam, M. A., Leal, I., & Ekramoddoullah, A. K. M. (2012). *Expressed sequences from the laminated root rot pathogen* Phellinus sulphurascens. Manuscript in preparation.

Williams, R. E., & Leaphart, C. D. (1978). A system using aerial photography to estimate area of root diseases centers in forests. *Canadian Journal of Forest Research, 8*, 214-219.

Wilson, J. S. (1998). *Wind stability of naturally regenerated and planted Douglas-fir stands in coastal Oregon, Washington, and British Columbia* (Ph.D. thesis). College of Forest Resources, University of Washington, Seattle, WA.

Winder, R. S., & Shamoun, S. F. (2006). Forest pathogens: Friend or foe to biodiversity? *Canadian Journal of Plant Pathology, 28*, S221-S227.

Winter, L. E., Brubaker, L. B., Franklin, J. F., Miller, E. A., & DeWitt, D. Q. (2002). Initiation of an old-growth Douglas-fir stand in the Pacific Northwest: A reconstruction from tree-ring records. *Canadian Journal of Forest Research, 32*, 1039-1056.

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications, 1*(5), 1-9. doi:10.1038/ncomms1053

Wymore, A. S., Keeley, A. T. H., Yrurralde, K. M., Schroer, M. L., Propper, C. R., & Whitham, T. G. (2011). Genes to ecosystems: Exploring the frontiers of ecology with one of the smallest biological units. *New Phytologist, 191*, 19-36.

Zaffarano, P. L., McDonald, B. A., & Linde, C. C. (2009). Phylogeographical analyses reveal global migration patterns of the barley scald pathogen Rhynchosporium secalis. *Molecular Ecology, 18*, 279-293.

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Zamani, A., Sturrock, R., Ekramoddoullah, A. K. M., Wiseman, S. B., & Griffith, M. (2003). Endochitinase activity in the apoplastic fluid of *Phellinus weirii*-infected Douglas-fir and its association with over wintering and antifreeze activity. *Forest Pathology, 33*(5), 299-316.

Zamani, A., Sturrock, R. N., Ekramoddoullah, A. K. M., Liu, J. J., & Yu, X. (2004). Gene cloning and tissue expression analysis of a PR-5 thaumatin-like protein in *Phellinus weirii*-infected Douglas-fir. *Phytopathology, 94*, 1235-1243.

Zhang, Y.-Y., Fischer, M., Colot, V., & Bossdorf, O. (2013). Epigenetic variation creates potential for evolution of plant phenotypic plasticity. *New Phytologist, 197*, 314-322.

Zhu, T., Kim, S.-H., & Chen, C.-Y. (2008). A medicinal mushroom: *Phellinus linteus. Current Medicinal Chemistry, 15*, 1330-1335.

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Appendix 1

Committee to Identify Approaches and Opportunities Ripe for Research on Understanding and Managing Root Diseases of Douglas-Fir

R. James Cook, Committee Chair, is best known for his research at Washington State University (WSU) on biological and ecological approaches to manage root diseases of wheat. He served at WSU as a research plant pathologist with the U.S. Department of Agriculture, Agricultural Research Service (ARS) for 33 years, starting in 1965. From 1993 to 1996, he headed the USDA's $100 million National Research Initiative Competitive Grants Program while maintaining his research program at Pullman. From 1998 to 2003, he held the R. J. Cook Chair in Wheat Research, a position endowed with a $1.5-million gift to WSU from the Washington Wheat Commission. He then assumed the duties of interim dean of the WSU College of Agricultural, Human and Natural Resource Science for two years before retiring in late 2005. In early 2006, the WSU Board of Regents named WSU's newest research farm the R. James Cook Agronomy Farm, in recognition of his career-long leadership in applied crop and soil management. He served as president of the American Phytopathological Society in 1983/84, the International Society for Plant Pathology from 1985 to 1993, and the Washington State Academy of Sciences in 2010/11. He has co-authored two books on biological control of plant pathogens and one book on wheat health management, and more than 200 research publications on plant diseases and crop management. He was elected to the U.S. National Academy of Sciences in 1993 and the U.S Agricultural Research Service Science Hall of Fame in 1997. He holds B.S. and M.S. degrees from North Dakota State University, a Ph.D. from the University of California, Berkeley, and honorary doctorates from North Dakota State and the University of Turin in Italy. In 2011, he was named co-winner of the Wolf Prize in Agriculture, awarded in Israel in late May in a ceremony at the Israeli Knesset with the presentation made by President Shimon Peres. rjcook@wsu.edu , www. planthealthinternational.com/

Robert L. Edmonds, Professor Emeritus, School of Environmental and Forest Sciences, University of Washington Seattle, WA, USA, holds a B.S. degree in forestry from Sydney University and the Australian Forestry School, and M.S. and Ph.D. degrees in forest pathology from the University of Washington, Seattle, Washington. He is known for his research on Pacific Northwest tree root diseases, aerobiology, mycorrhizae, decomposition, nutrient cycling, and watershed and ecosystem ecology. He spent 36 years on the faculty at the University of Washington, including 10 years as Associate Dean for Research in the College of Forest Resources,

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before retiring in 2012. He has published more than 150 articles, including 75 peer reviewed journal articles, and three books (*Aerobiology: the ecological systems approach; Analysis of Coniferous Forest Ecosystems in the Western United States; and Forest Health and Protection*). He is an Honorary Life Member of the Northwest Scientific Association for service to the Society. He also received an Outstanding Teaching Award from the College of Forest Resources, Alumni Association, and a Lifetime Achievement Award from the Western International Forest Disease Work Conference. He has served on two National Research Council Committees. bobe@uw.edu

Ned B. Klopfenstein, (USDA Forest Service, Rocky Mountain Research Station, Moscow, ID, USA) has served as a Research Plant Pathologist for more than 20 years. He is best known for his use of genetic tools to address diverse issues in forest pathology. His early career focused on molecular genetics of Populus spp., during which he led the first field test of genetically engineered Populus, edited the book *Micropropagation, Genetic Engineering, and Molecular Biology of Populus*, and examined the molecular genetics of host-pest interactions. His subsequent studies have addressed molecular diagnostics and phylogenetic relationships of forest pathogens, population genetics of forest hosts and pathogens, predicting impacts of climate change on forest disease, and gene expression of forest pathogens. His research, which involves several international collaborations, has focused on *Armillaria* root disease, white pine blister rust, fungal endophytes of forest trees, *Fusarium* disease of conifer seedlings, and invasive guava-eucalypt rust pathogens. These studies have resulted in more than 100 publications to date. nklopfenstein@fs.fed.us

Willis Littke, Forest Scientist, Weyerhaeuser Company, Federal Way, Washington, USA, has an M.S. from Western Washington University, Bellingham, and a Ph.D. in forest pathology from the University of Washington, Seattle. He conducts research on forest and nursery pathology, and other forest health problems in plantations. will.littke@weyerhaeuser.com

Geral McDonald, (Scientist Emeritus, USDA Forest Service, Rocky Mountain Research Station, Moscow, ID, USA) served as a Research Plant Geneticist, Research Plant Pathologist and Principle Plant Pathologist from 1966 to 2003 at the Moscow Forestry Sciences Laboratory. He received a B.S. degree in Forest Management (1963) and a Ph.D. in Plant Pathology and Genetics (1969) from Washington State University. He applied classic quantitative genetics, ecology, and molecular genetics to the study of forest diseases and insects. He is best known for using principles of

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ecological genetics and community ecology to study introduced white pine blister rust (*Cronartium ribicola*) and all North American species of the fungal genus *Armillaria*. This genus resides in the soil and includes decomposer, pathogenic, and perhaps mutualistic species. Species of *Armillaria* produce extensive networks of rhizomorphs that connect to the roots of shrubs and trees and to detritus in all but the driest forested ecosystems of North America. He has authored and coauthored more than 100 publications ranging from book chapters to refereed journals. geral.mcdonald4@gmail.com

Daniel Omdal, Forest Health Program, Washington State Department of Natural Resources, Olympia, WA, is the State Forest Pathologist for Washington. He received a B.S. degree in biology from Houghton College, an M.F. in forestry from Duke University, and a Ph.D. in forest pathology from Colorado State University. He serves as the senior program expert on issues relating to diseases of forest trees and has lead responsibility for planning and directing activities statewide designed to protect 12 million acres of state and private forest lands against destructive pathogens. daniel.omdal@dnr.wa.gov

Karen Ripley, Recording Secretary for the Study Committee, Forest Health Program, Washington State Department of Natural Resources, Olympia, WA, is a forest entomologist who also manages the state's Forest Health Program. She received a B.A. degree in biology from Whitman College, Walla Walla, WA, and an M.S. degree in forest entomology from the University of Washington, Seattle, WA. She provides technical assistance regarding forest insects and health to state and private forest landowners and managers. She has contributed articles and training sessions to many forest landowner publications and educational programs, annual WDNR Forest Health Highlights, and agency strategic management plans including "Washington's Strategic Plan for Healthy Forests," "A Desirable Forest Health Program for Washington's Forests," and "A Wildland Fire Protection Program for Washington." karen.ripley@dnr.wa.gov

Terry Shaw, Charles G. "Terry" Shaw is a retired Research Plant Pathologist with nearly 40 years of experience with both the New Zealand and U.S. Forest Services. He received a bachelor's degree in forestry from Washington State University in 1970 and a Ph.D. in plant pathology from Oregon State University in 1974. Dr. Shaw's specialty has been with root diseases of forest trees, especially *Armillaria* root disease, on which he has published numerous scientific articles. In the 1990's, He compiled the U.S. Department of Agriculture Handbook on *Armillaria* root disease, bringing authors together from around the world to complete this treatise.

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Terry also spearheaded the effort to develop a predictive computer model on the behavior, environmental impacts, and economic losses from root diseases in the forests of western North America. The resulting Western Root Disease Model is in operational use today. cgsarchxx@aol.com

Rona Sturrock, (Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, Canada) is a research scientist working in the field of forest pathology. She received her B.S. in biology from the University of Victoria in 1983 and her Master of Pest Management (MPM) degree from Simon Fraser University, Burnaby, British Columbia, in 1989. From 1986 to 1988, she conducted research on forest pathogens of nursery-grown coniferous seedlings and helped provide extension services in this area. From 1989 to 1990, she was seconded to a program assisting BC First Nations develop and manage their forest resources. Starting in 1991 and continuing until quite recently, she conducted research on the fungus *Phellinus sulphurascens* and the disease it causes, laminated root rot (LRR). Rona joined forces with USDA Forest Service researcher Walt Thies in 1995 to publish a guide titled "Laminated Root Rot in Western North America," which continues to be widely used. Rona's research papers, reports, and guides on LRR cover aspects of disease management; the biology and diversity of *P. sulphurascens*; and molecular aspects of the *P. sulphurascens*-Douglas-fir interaction, including Douglas-fir resistance to the fungus. More recently, Rona has investigated and reported on forest diseases and climate change. Her publications on this variety of subjects number approximately 50.

Paul Zambino, plant pathologist, USDA Forest Service, Forest Health Protection, Coeur d'Alene, ID, USA, has contributed to advancements in plant pathology and understanding of host-parasite interactions and fungal diversity for more than 20 years. He has developed techniques of screening for disease resistance in horticultural, ornamental and tree species, and is best known for his improvements to North American screening programs that detect resistance to white pine blister rust in white pine species. In his early career, he published the first ribosomal ITS and further developed crosses of wheat stem rust to study inheritance and enable genetic mapping of rust avirulence genes by molecular methods. He has also studied fungal genetic diversity in Ustilago, Coprinus, and Cronartium using molecular markers. In his current position in Forest Health Protection, he is involved in activities aimed at advancing the ability of forest managers to identify and counteract forest health problems in forests of the Inland Northwest. Root diseases are the greatest challenge to forest health in many of these forests. Restoration and reestablishment of root disease-resistant western white pine and white bark pine that have

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been decimated by the combined effects of blister rust, altered management and fire exclusion will be critical in affected ecosystems, so his current interests are to examine factors that contribute to location-specific risks of white pine blister rust and test for optimize activities to minimize these risks and enable plantings and regeneration to restore dysfunctional ecosystems to health. He holds a B.S. degree from Gustavus Adophus College, MN, an M.S. from the University of Minnesota, and a Ph.D. from the University of New Hampshire.

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Appendix 2

Department of Natural Resources Request for the Report and Formation of the Committee

In correspondence received September 2, 2011, the Washington State Department of Natural Resources (DNR) requested help from the Washington State Academy of Sciences (WSAS) to

> evaluate which among the full complex of root diseases [of Douglas fir] is appropriate for further scientific research toward applied management options . . . [and to focus on] identifying and implementing molecular research on the host-pathogen relationship between Douglas-fir and root diseases such as laminated root rot [caused by] (*Phellinus sulphurascens*) and *Armillaria* root disease.

The WSAS advises state agencies on the use of science and engineering for priority setting and policy making but does not do (implement) research. Instead, and similar to the process used by the National Research Council, the WSAS upon agreeing to undertake a project such as requested by the DNR will develop a statement of task in consultation with area experts and DNR leadership, assemble a committee that would bring relevant experts and information together, and produce a written report of findings and recommendations. Only the chair of this committee needs to be a member of the WSAS. The resultant report presumably would be useful to federal agencies such as the U.S. Forest Service and National Science Foundation and to private industry as well as the DNR in funding and implementing the research priorities identified. The Committee that conducted this study and produced this report is shown in Appendix 1.

Not forgoing the idea of a workshop, the September 2 letter from the DNR Commissioner of Public Lands included a second request of the WSAS, namely to organize and conduct "practical field forester-level training on disease identification and application of known management strategies." While no further action has been taken to organize "practical field forester-level training," the Findings and Recommendations in this report include examples of known management strategies available for immediate application. Such a workshop should include segments on identification, documentation, and management of LRR and new developments in molecular biological approaches that are particularly promising for future understanding and improved management of LRR.

It was fortuitous that the Western International Forest Disease Work Conference was scheduled to meet October 10-14, 2011, in Leavenworth, Washington. To take advantage

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of this opportunity, the DNR and WSAS co-hosted an evening discussion on October 12, co-chaired by DNR State Forester Aaron Everett and Cook. Thirty-two members of the conference attended the discussion session. Out of this group, eight–a number later increased to nine–agreed to join Cook as chair (Appendix 1) of a new WSAS study committee that would develop a statement of task, conduct the study, and write the report on approaches and opportunities for research on understanding and managing root diseases of Douglas-fir. Of the eight forest pathologists and one forest entomologist named to this study panel, three were from the U.S. Forest Service, one from Oregon and two from Idaho; one was from Natural Resources Canada; two represented the University of Washington and University of Idaho, respectively; two were from the DNR; and one was from Weyerhaeuser. These nine members of the study committee represented a combined 344 years of experience. Cook, serving as chair, has 48 years of experience working on root diseases and soilborne plant pathogens of agronomic crops.

Along with the agreement between the WSAS and DNR to form this study committee, it was decided by the WSAS Executive Committee that the cost of conducting this study and publication of the report would be funded with appropriations provided to the WSAS by the legislature, with the DNR responsible for expenses incurred by DNR in the course of the study, e.g., travel of DNR staff. Because state funds appropriated for work of the WSAS are held in the respective budgets of Washington State University and the University of Washington, travel and other costs of conducting this study and producing this report were approved and handled as expenses billed to Washington State University or University of Washington.

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Appendix 3

Procedures Used to Reach Consensus on Preliminary Findings

Following the evening discussion session held October 12, 2011, as part of the Western International Forest Disease Work Conference, the Study Committee began its work over the next four months using email to exchange ideas on research needs and opportunities across the wide range of forest root diseases. The substance of these email discussions then served as the basis for discussions and approval of a statement of task, and for subsequent discussions and conclusions at a meeting held February 16-17, 2012, in Seattle (Appendix 4). All nine members of the Study Committee participated in both days of the meeting, two by phone and eight in person at WSU-West in Seattle. Writing assignments were made at the February meeting in Seattle with a goal to assemble a first draft in about six months, allowing for the season when forest pathologists and entomologists are in the field. After successive exchanges of drafts, the Committee met November 16 and 17, 2012, at WSU-West in Seattle for a final discussion and agreement of the findings and recommendations.

The statements and conclusions below summarize the preliminary findings and consensus reached by the Committee by the end of the February meeting in Seattle and further refined at the November meeting in Seattle. A key outcome of the February meeting was the decision to focus this study and report on LRR of Douglas-fir caused by *Phellinus sulphurascens*.

1. Losses due to root diseases, namely LRR caused by *Phellinus sulphurascens*, *Armillaria* root disease caused by any one of several species of *Armillaria ostoyae*, and annosus root and butt rot caused by *Heterobasidion annosum* (now *H. occidentale*) may be due as much or more to retardation or limitations of volumetric tree growth as to outright killing of trees such as Douglas-fir (Buckland et al., 1954; Bloomberg & Wallis, 1979; Tkacz & Hansen, 1982; Lawson et al., 1983; Thies, 1983; Cruikshank et al., 2011).
2. The loss of (shortfall in) growth due to root diseases of Douglas-fir and other timber species may be a factor along with insect damage in accounting for why Northwest forests are shifting from being sinks to sources of carbon dioxide at a time when forests are needed for more not less net carbon sequestration (Cohen et al., 1996; Post et al., 2012).
3. Whereas insect outbreaks occur annually on thousands of hectares, the best estimates are that root rots occur significantly on 5-15% of the land base, with LRR causing the greatest loss of coastal Douglas-fir.

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4. The primary justification for the DNR to limit losses from root diseases on State-owned forest land is protection of timber production in accordance with the DNR mandate that state timber lands be managed for income to support schools and other trust beneficiaries, while recognizing the importance of environmental services and helping to assure an adequate supply of timber for the built environment. The same justifications would apply to privately owned and managed timberland. Even landowners who do not focus on timber production management objectives can be impacted when infected trees are a hazard to roadways and developed recreation sites. And management for wildlife can be affected by shorter duration as standing snags of root disease-killed trees that reduces their effectiveness as nesting sites. All landowners may need to focus disease management on their highest productivity sites with the highest potential for valuable timber production or their most valuable developed sites.

5. With the growing interest in and federal funding for development of the technology and supply chain to produce jet fuel for commercial and military aircraft in the Pacific Northwest using residual woody biomass (www.nararenewables.org/Cached), loss of growth potential of trees due to root diseases represents a loss in the potential for coniferous woody biomass to become another source of revenue for the DNR and private landowners and contribute to the national goal of sustainable alternatives to fossil fuels.

6. The research approaches and tools needed to address root diseases must address a heterogeneous physical landscape that interacts with a complex biological system (host/pathogen/associated soil and rhizosphere microbiota), creating a near-infinite number of unique ecological situations that change temporally; and that a single stand (the customary management unit in forestry) may encompass three or four eco-evolutionary histories, each requiring a unique prescription or customized approach for management that could vary from no action to stand replacement.

7. Some of the most devastating pathogens of trees such as those responsible for white pine blister rust, Dutch elm disease, and chestnut blight were introduced into the United States with the introduction of their hosts as trees or other plant material. In contrast, all evidence indicates that the root pathogens of Douglas-fir and other timber species in Washington forests are a natural component of Pacific Northwest forest ecosystems. In some cases, the age of a single *Phellinus* clone has been estimated to be around 1,000-2,000 years, indicating a long period of association/adaptation with multiple forest communities.

8. *Phellinus* does not survive well away from the roots or other woody material colonized through parasitism, but its transition from parasitism to saprophytism

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as the tree dies offers the opportunity to determine through transcriptomics what genes are involved during these two contrasting phases of its life cycle.

9. After old-growth forest was harvested, large *Phellinus*-infected stumps were potentially massive "havens" for inoculum capable of supporting the pathogen for at least one and possibly several hundred years into the following rotation. On the other hand, succeeding rotations are producing smaller and smaller residual stumps in which the pathogen will survive for less time. Although clone survival of *P. sulphurascens* may be challenged as residual stump size continues to decrease with shorter rotations, this effect may be ameliorated by the greater number of infected stumps in these rotations compared with old-growth stands.

10. The tools of metagenomic and transcriptomic analyses provide the means to study processes by a data-driven approach from which hypotheses can be derived and tested.

11. Research on the transcriptome of LRR in a managed forest and under diverse environments can reveal expressed genes without regard to whether they are plant or pathogen genes or part of an associated microbiota, e.g., endophytes or of a microbiome more broadly.

12. Research using the tools of metagenomics and transcriptomics will be complemented with simplified field and small-plot studies to discern or confirm indicated biological processes and relationships.

13. The best metagenomics analyses are coupled with careful, detailed environmental monitoring so the conditions and processes can be discerned.

14. Forest soils are a promising area for a metagenomic study because there is a high likelihood that increased knowledge of biological processes will improve land management today and benefit climate adaptation and carbon storage goals in the future.

15. Traditional forest pathology research is changing because of the demographics of current researchers (retiring) and shifts in funding priorities in research organizations (universities, U.S. Forest Service, Natural Resources Canada) away from plant pathology. As with other areas of biological and environmental research, there is a shift toward greater use of molecular approaches and multidisciplinary teams.

16. There is an urgent need in the study and control of forest tree diseases generally and root diseases more specifically to attract disciplines outside of traditional field of forest pathology to get involved in this research.

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Appendix 4

Committee Meeting Agendas

First Meeting Agenda

WSU-WEST (520 Pike St., Suite 1101, Seattle, WA)

February 16, 2012, 1 to 5 p.m.

February 17, 2012, 8 a.m. to noon

- Introductions / housekeeping
- Review of goals of the meeting
- Review of draft Statement of Task

  The Committee will determine which among the full complex of forest tree root diseases is appropriate for further scientific research toward applied management options, with a focus on [identifying and implementing] molecular research on the host-pathogen relationship between Douglas-fir and root diseases such as laminated root rot (*Phellinus sulphurascens)* and Armillaria root disease.

- Discussion of potential target(s) [tree root pathogen(s)] of the study
  - *Armillaria* spp.
  - *Phellinus* spp.
  - *Heterobasidion* spp.
- Current knowledge [or limits to knowledge] of the range and ecology of:
  - *Armillaria* root rot of Douglas-Fir
  - laminated root rot of Douglas-Fir
  - *Heterobasidion* root rot of Douglas-Fir
- Review of current "applied management options" and their limitations/costs
  - *Armillaria* root rot of Douglas-Fir
  - laminated root rot of Douglas-Fir
  - *Heterobasidion* root rot of Douglas-Fir

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- What can we expect to learn from molecular research on host-pathogen relationships that would "further scientific research toward applied management options"?
    - *Armillaria* root rot of Douglas-Fir
    - laminated root rot of Douglas-Fir
    - *Heterobasidion* root rot of Douglas-Fir
- Discussion of areas proposed as ripe for research

Second Meeting Agenda

WSU-WEST (901 5th Street, Seattle)

1 to 5 p.m., November 26

and 8:30 a.m. to 12:30 p.m., November 27, 2012

- Introductions of Laurel le Noble and Jane Stewart
- Comments from Laurel on her roles and expectations of the Committee
- General Discussion of the Report: Organization, Completeness, etc.
- Review and strengthening of content related to molecular research
- Discussion/corrections section by section
- Further writing assignments, if needed
- Suggestions/assignments for photographs
- Suggestions for outside reviewers
- Time-frame for report to the WSAS Report Review Committee
- Wrap-up

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