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# Using chemical markers to detect root disease in stressed ponderosa pine stands with a low incidence of disease in eastern Oregon

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**Abstract**

A total of 284 ponderosa pine growing near the southern edge of the Blue Mountains in eastern Oregon were categorized into one of three crown classes based on the degree of "tufted", or "lion's tail" appearance of their branches, a potential symptom of black-stain root disease, then pushed over and their root systems examined for visual symptoms of disease. Only 23 (8.1%) were found to have more than a trace level of black-stain, annosum root disease, or both at about 38 cm below the root collar. Although there were many measurable differences in crown growth parameters among the three classes, they were not reliable for predicting the presence, or severity of root disease. Differences in crown morphology associated with these classes probably resulted from the combined effects of tree size, levels of resources available on site to support vigorous growth, especially water, and stresses that enhanced water deficits. The sapwood water content of diseased trees was 80.0% of the water content in healthy trees. Acetaldehyde, acetone, methanol, and ethanol concentrations, quantified in the headspace analysis of sapwood collected above the root collar prior to harvest, were all higher in trees with root disease compared to those without disease. Logistic regression models with 0–5 chemical explanatory variables were compared simultaneously by the practical information-theoretic approach to select the best model for predicting trees with root disease. The one selected contained only acetone fresh weight concentrations as an explanatory variable, and would facilitate increased rates of sapwood analysis in the laboratory. More importantly, acetone concentrations may function as useful markers to identify the most severely diseased trees for removal, or in general stand surveys to estimate the level of root disease when symptoms in the crown are lacking or confounded by other stresses.
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*Keywords:* *Pinus ponderosa*; Blue Mountains; Predicting root disease; Headspace chromatography; Acetone; Acetaldehyde; Ethanol; Water content; Biomarkers; Black-stain root disease; Annosum root disease

## 1. Introduction

Root disease is a common cause of death and physiological stress for conifers in western forests of North America. It is typically caused by one or more species of fungi, the principal pathogens being *Phellinus weirii* (Murr.) Gilbertson, *Armillaria ostoyae* (Romagnesi) Herink, *Heterobasidion annosum* (Fr.) Bref., and *Leptographium wageneri* (Kendrick) J.J. Wingfield. Patches of dead trees or infection centers are helpful indicators that a pathogen is present, and close inspection of the dead trees can often provide sufficient clues to identify the causal organism (Hagle et al., 2003). However, verifying a root pathogen in live trees can be more difficult (Hansen and Goheen, 2000), especially in young trees, or when the infected individuals are scattered across a stand or landscape, and may be stressed simultaneously by others agents such as drought, or insects. While root diseases do have positive and beneficial impacts on forest ecosystems, they cause changes in community composition, structure, or diversity, with unexpected and occasionally undesirable consequences for established management objectives, whether ecological or economic in context. To more accurately predict how root pathogens will impact management prescriptions over the long term, Hansen and Goheen (2000) advocate improvements in existing root disease models. However, they identified the lack of information describing current root disease conditions, caused largely by the difficulties of accurately identifying infected trees, as the

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greatest challenge associated with model improvements. Omdal et al. (2004) found that using a root disease rating system (Stage et al., 1990) based on tree symptomatology and proximity to confirmed infected trees and stumps was better at identifying infected trees (Douglas-fir 14/22, white fir 26/28, and ponderosa pine 74/79) than it was at correctly identifying those without disease (7/14, 2/18, 2/18, respectively).

Visible crown symptoms may or may not be expressed in response to infections by root pathogens (Filip, 1986; Omdal et al., 2004; Worrall et al., 2004). Laminated root rot caused by *P. weirii*, is notorious for showing minimal crown symptoms (Thies and Sturrock, 1995). For ponderosa pine, *Pinus ponderosa* Doug. Ex Laws., in eastern Oregon infected with *L. wageneri var. pondersoum* (T.C. Harrington & F.W. Cobb) T.C. Harrington & F.W. Cobb (black-stain root disease) or *H. annosum* (annosum root disease), or both, the crown symptoms do not appear until the infection reaches moderate to severe levels with 61.2 and 85.5% of the total cross-sectional root area being non-functional at 30 cm distance from the root collar, respectively (Kelsey et al., 1998). Black-stain is a wilt disease that disrupts water transport in the host (Hessburg, 1984; Hessburg and Hansen, 1987; Joseph et al., 1998), and contributes to symptom development in the crown because of the relationship between water supply and tissue growth. For example, artificially limiting water supplies to healthy red pine, *Pinus resinosa* Ait, causes decreased bud size and reduced shoot elongation compared to those trees supplied with ample water (Clements, 1970).

We speculate that at least one-third or more of the cross-sectional root area at 30–40 cm from the root collar must be diseased and non-functional in ponderosa pine before growth in the crowns is impacted substantially. Crown parameters of trees with light root disease (15.2% non-functional root area) were similar to healthy trees, whereas those with moderate disease (61.2% non-functional root area) expressed substantial reductions in growth of the terminal leader and lateral branches; 34.2 and 39.7% of the healthy trees, respectively (Kelsey et al., 1998). Thus, crown symptoms begin to appear as the non-functional root area increases from 15.2 to 61.2%, possibly around their mid-point (38.2%). Reductions of this magnitude are probably necessary because the root systems of healthy conifers have excess capacity for water uptake, and can tolerate substantial loss without greatly diminishing water transport, provided they are not stressed by drought. For example, Teskey et al. (1985) mechanically severed approximately one-third of the root system from Pacific silver fir, *Abies amabilis* (Dougl.) Forbes, during the summer and found no effect on sap velocity or foliar water relations. Ponderosa pine with moderate and severe root disease expressed reduced branch and terminal growth and increased needle density near the branch tip giving them a "tufted," or "lion's tail," appearance, and the previous year's growth of lateral branches was the best single crown parameter for predicting disease trees using logistic regression (Kelsey et al., 1998). However, use of these crown symptoms to identify diseased trees requires additional evaluation.

Chemical markers from ponderosa pine sapwood may have some value for identifying trees with root disease (Kelsey et al., 1998). Acetaldehyde, acetone, methanol, 2-propanol, and ethanol concentrations in samples of stem sapwood collected just above the root collar, in each cardinal direction, were measured by headspace gas chromatography. All compounds, except ethanol, were more concentrated in trees of the moderate and severe disease classes, than those in the healthy and light diseased classes. Acetaldehyde concentrations increased incrementally with increasing disease severity and it was determined by logistic regression to be the best single compound for predicting diseased trees, and was also better at predicting disease than the prior year lateral branch growth (Kelsey et al., 1998).

The objectives of this study were: (1) to further evaluate the appearance of "lion-tails" in crowns of eastern Oregon ponderosa pine for use as a key indicator of root disease, particularly black-stain, when surveying to locate scattered individuals or to determine the extent of infection centers; (2) to further evaluate the potential for using headspace chemical markers as predictors of root disease in ponderosa pine.

## 2. Methods and materials

### 2.1. Study site

The study area (centered at 43°45.92′N; 118°56.11′W) is located near the south end of the Blue Mountains (ranging from approximately 1646 to 1768 m elev.) on the Malheur National Forest, about 21 km northeast of Burns, Oregon (Fig. 1). Eight stands of ponderosa pine within a 2.0 km radius of the center, above, were selected for study. Pine with a diameter at breast height (DBH) $\geq$ 7.62 cm had a mean basal area of 26.86 $m^2$/ha, with a mean of 13,708 seedlings/ha (DBH < 7.62 cm). Soil depths range from a low of 20 to 30 cm and a high of 38 to 76 cm, with 10 to 60% of the surface covered with flat rock fragments. The soil is a loam, gravely loam, or clay loam material with gravel and cobble content increasing with depth and ranging from 20 to 60% (Carlson, 1974). The bedrock is

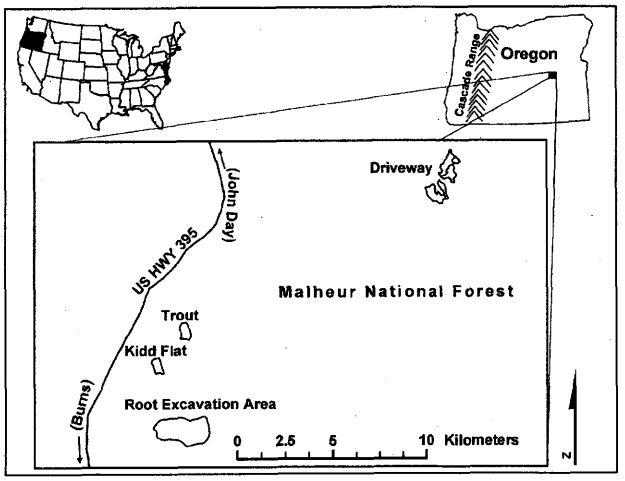


Fig. 1. Location of the root excavation study area, and the nearby study sites of Thies et al. (2005) at Kidd Flat, Trout, and Driveway.

moderately hard to hard rhyolite, or hard basalt and andesite with some tuffaceous interflow material. The growing season is short, with hot summer days and cool to cold nights. Annual precipitation is low to moderate, averaging from 38 to 51 cm (Carlson, 1974, isohyetal map) with the majority as winter snowfall. All stands were scheduled for commercial thinning within the year, and those we harvested were part of this thinning.

### 2.2. Selecting sample trees

A series of parallel north–south transect lines, separated by 40 m, were established across each stand, with centers for variable plots marked at 40 m intervals along each line. Trees ranging from 7.6 to 52.5 cm were selected at each variable plot center by observing them at breast height through a 10 BAF prism. The upper one-third of their crowns was carefully observed for the appearance of lion's tailed branches, a potential indicator of root disease (Cobb, 1988; Kelsey et al., 1998). Crowns were categorized into one of three classes, based on the most abundant branch type observed: (class 1) strong lion's tail appearance, with needles primarily on the terminal 5 cm or less of the branch; (class 2) moderate lion's tail appearance, with needles primarily on the terminal 15 cm of the branch; (class 3) no lion's tail, the branches retained needles on more than the terminal 15 cm and appeared to have a full compliment of normal length needles like nearby healthy-appearing trees.

The next task was to randomly select three groups of 100 trees: (1) potentially healthy from crown class 3; (2) potentially diseased from crown class 1 or 2; (3) the nearest neighbor of a potentially diseased tree regardless of crown classes. This was achieved by first dividing all the plots into two groups: (1) healthy plots—those plots with all trees in crown class 3; (2) potentially diseased plots—those with at least one tree in crown class 1 or 2. One hundred plots were randomly selected from each group. On each healthy plot, one crown class 3 tree, less than 52.5 cm dbh (trees $\geq$ 52.5 cm could not be cut) was selected randomly and tagged for sampling. On each potentially diseased plot, one crown class 1 tree was selected randomly and tagged. If a class 1 tree was not present, then a crown class 2 tree was selected randomly. Additionally, on each potentially diseased plot, the nearest neighbor of each selected tree, regardless of its crown class, was tagged and included in the group of 100 trees selected randomly from all three crown classes. Diameters of these tagged trees were measured at breast height (1.37 m above the forest floor on the up hill side), and sapwood samples were collected near the root collar for chemical analysis. These trees were then pushed over to excavate their roots for cleaning and evaluation of disease severity.

### 2.3. Sapwood sampling

From 5 to 9 June 2000, samples of sapwood (5 mm $\times$ 50 mm) from near the root collar (1 to 5 cm above the litter layer) were collected from the four cardinal directions on each tagged tree using a battery powered drill and increment borer bit. Each sample was individually sealed in a glass tube (12.6 mm $\times$ 100 mm o.d.) with a screw cap and immediately frozen with dry ice. They remained frozen in transit to the laboratory where they were stored in a $-36$ °C freezer until analyzed.

### 2.4. Headspace analysis of sapwood

Frozen samples were adjusted to about 4 °C with ice then weighed into preweighed headspace vials and sealed with a septum. The vials were heated at 102 °C for 30 min and analyzed for headspace volatiles by gas chromatography (GC) using the same instruments and settings as described previously (Kelsey and Joseph, 1998), except the sample thermostat time for the Perkin-Elmer HS40 autosampler was increased from 20 to 30 min. Also, the column oven on the gas chromatograph was programmed with a starting temperature of 50 °C for 0.5 min, and then increased to 80 °C at 20 °C $min^{-1}$ with a 1.5 min hold. The total run time was 3.5 min. Each vial was analyzed twice with venting between runs as required for calculating tissue concentrations using multiple headspace extractions (MHE; Kolb et al., 1984). The standard solution used to calibrate the instrument contained acetaldehyde, acetone, methanol, and ethanol diluted in deionized water. As observed previously (Kelsey et al., 1998), the acetone and methanol in sapwood samples were partially or entirely generated during heating, so their concentrations were estimated from peak heights in the first chromatogram rather than by MHE. 2-Propanol was also present, but not quantified as it previously appeared to have less value as a biomarker for root disease than the other compounds above (Kelsey et al., 1998). Compound concentrations in the four core segments were averaged to give a mean tree value for statistical analysis. Sapwood dry weights were measured by removing septa from the analyzed vials then heating for 16 h at 102 °C, cooling to room temperature in a desiccator box, and weighing.

### 2.5. Sampling crowns and roots

Between 11 September and 5 October, the tagged trees were pushed to the ground with a blade on the front of either a bulldozer or rubber tired skidder. The terminal leader and two lateral branches from opposite sides of the crown near the terminal were clipped and taken to a walk-in cooler each night for storage until all trees were processed. The root end of the tree was lifted off the ground with a 2 prong grapple and transported to a processing site where the root system was removed and a thin disk cut from the lower stem for estimating age. All soil was cleaned from around each root system with hand tools. Terminal ends of the roots were clipped and examined for any evidence of resinosis, rot, or staining, and their presence noted if found. All roots were then cut at approximately 38 cm (15 in.) from their point of attachment to the bole and examined for resinosis, rot, or stain. Roots broken during extraction from the ground, and less than 38 cm long, were cut at a distance allowing the maximum cross-sectional

area remaining to be observed. When all roots on a tree were healthy their functionality was recorded as 100%. If more than a trace of resinosis, rot, or stain was found in roots ≥5 cm (2.0 in.) diameter at 38 cm from the stem, then a 1–3 cm thick disk was removed from the end of all roots this size. The root disks were placed in a plastic bag and taken with the stem disks to a walk-in cooler for storage at the end of each day. After processing all trees, the crown, stem, and root samples were transported to the laboratory for further measurements.

### 2.6. Estimating root disease

This procedure and disease symptoms noted were the same as used previously (Kelsey et al., 1998). Transparent plastic sheets were placed over the root disks and the areas marked based on visible symptoms as follows: healthy—white colored tissues; resinosis—brownish-black areas that appeared wet or resin soaked; brown-stain—based on previous observations where this color was associated with yellow, fibrous decayed wood typical of *H. annosum*; black-stain—long narrow bands of black colored wood, often near the sapwood perimeter in a crescent shape, previously identified as *L. wageneri* var. *pondersoum* by culturing (Kelsey et al., 1998); or other—primarily blue-stain from *Ophiostoma* spp. appearing as a bluish-grey triangular patch, with the apex pointing toward the root center. The latter is not considered a root disease, but was recorded since it does impact root function. The tracings from all roots of a tree were cut out and sorted by category, then weighed to estimate their proportion of the total. Only the area identified as healthy was assumed to be functionally capable of water conductance as demonstrated by Joseph et al. (1998).

### 2.7. Crown measurements

Previous years shoot growth, or internode length, was measured for the terminal and two lateral leader branches as in our earlier study (Kelsey et al., 1998). Additional measurements for the two lateral branches included diameter of the previous years internode (taken midway), number of needle fascicles on the previous years internode, fascicle density on the previous years internode (surface area calculated from length and diameter), number of years growth (internodes) retaining five or more needle fascicles, and total length of the branch retaining needles measured from the branch tip.

### 2.8. Statistical analysis

All analyses were conducted using SAS version 8 or 9 (SAS Institute) with individual trees as the experimental unit. The various crown parameters, and concentrations of the four headspace compounds or sapwood water content were analyzed separately using analysis of variance (GLM) for a completely randomized design with treatment groups independently assigned as crown classes (1, 2, and 3), or by their root disease status (diseased or healthy). Normality and homogeneity of variance were checked prior to analysis for all data sets by examining normal probability plots, and plots of the residuals (observed versus predicted), respectively. Five of the crown parameters (terminal leader length, lateral leader length and diameter, lateral fascicle number, and lateral needle retention length) and the concentrations of all chemical components, except water content, were transformed with natural logarithms. The differences between means were compared by Fisher's Protected LSD (only when the model $p < 0.05$) and considered significantly different at $p \leq 0.05$. Transformed means and their 95% confidence limits were back-transformed to medians and associated confidence limits for presentation.

Using a macro program in SAS, 32 logistic regression models containing all combinations of 1–5 explanatory variables (the dry weight concentrations of four headspace compounds transformed as natural logarithms, and sapwood water content) and a model with no variables, were compared by the practical information-theoretic approach (Burnham and Anderson, 1998) to select the best model (with the lowest Akaike's information criterion, $AIC_c$) for predicting trees with root disease. The same models using transformed fresh weight concentrations of the four compounds, plus sapwood water content, were compared in a separate analysis. Burnham and Anderson (1998) emphasize the importance of carefully defining a subset of models to compare based on the objectives and what is know about the problem. Although acetaldehyde has been identified as a marker of root disease in ponderosa pine (Kelsey et al., 1998), the trees sampled in that study had not been randomly selected, and acetone, methanol, and water, like acetaldehyde, were also strongly correlated with the % non-functional root area caused by the disease. We felt our *a priori* information was not reliable enough to assist in eliminating explanatory variables or models, so we tested all 32.

Performance of the top six models selected using compound dry weight concentrations, and the top model using fresh weight concentrations were compared with the trees from the existing data set using Statgraphics Plus 4.0. Each model calculates a predicted probability of root disease for each tree in the data set then compares this value to a "cut-off" probability used to sort the trees. When the predicted probability is equal to or greater than the cut-off probability, the tree is identified as diseased, otherwise it is healthy. Model predictions are compared with the observed root disease conditions for each tree and the proportion of correct matches reported for diseased, healthy, and total trees categories. The correctly predicted proportions for each category are calculated for a series of 21 probability cut-off values ranging from 0 to 1, in increments of 0.05, allowing performance of the models to be easily compared. Statgraphics Plus 4.0 was used also for regression of chemical parameters with the % non-functional root area within the group of trees having root disease.

## 3. Results

The root systems from 284 of the original 300 tagged trees were extracted from the ground and examined for the presence of disease. The remaining 16 were not processed because their roots could not be extracted, or they were damaged excessively

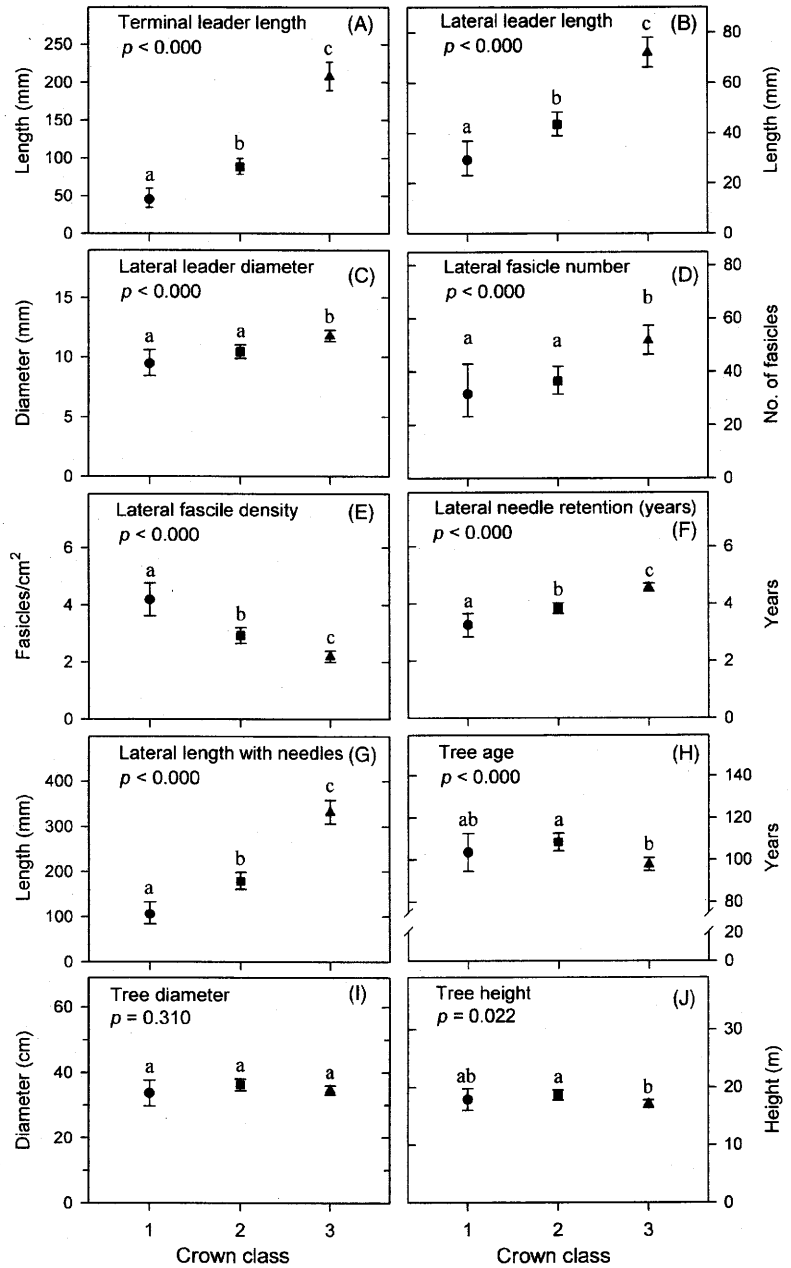


Fig. 2. Foliar and stem parameters measured for trees in each of the three crown classes. Graphs A, B, D, G show medians ± 95% CI, and all others show means ± 95% CI. $p$ Values are from the GLM analysis. Symbols with the same letters above are not significantly different at $p = 0.05$.

during removal. A total of 21, 95, and 168 trees were categorized as crown classes 1, 2, and 3, respectively. Most crown parameters were significantly different among the three classes (Fig. 2). Crown class 3 trees appeared healthy and grew more vigorous the previous year than trees in class 1 or 2. Class 3 trees were also younger and shorter than those in class 2, whereas class 1 trees were intermediate for both age and height. There were no differences in stem diameters among the three crown classes.

Only 23 of the 284 trees (8.1%) were found to have more than a trace level of root disease detectable at 38 cm from the bole (Table 1), with 8, 8, and 7 diseased trees categorized as crown classes 1, 2, and 3, respectively, which represented 38.1, 8.4, and 4.2% of the trees in each class. Diseased trees in crown class 1 had 55.1% (±35.1, S.D.) of their cross-sectional root area non-functional at 38 cm from the bole, compared with 41.0% (±34.6) for diseased trees in crown class 2 and 33.4% (±18.3) for those with disease in class 3. Trees in crown classes 1 and 2 were 3.5 times (95% CI 1.4–8.8 times) more likely to be diseased than the trees in class 3. Root diseases detected were probably annosum root disease and black-stain root disease, although culturing was not done to verify the causal fungi. When trees were categorized by their root disease status (Fig. 3), their crown parameters showed trends similar to the

Table 1
Percentage of the total root system cross-sectional area, at approximately 38 cm below the root collar, impacted by resinosis or disease in the trees found to be infected[a]

| Root disease or condition | Number of trees with condition | % Total root area with condition | | |
|---|---|---|---|---|
| | | Mean (±S.D.) | Maximum | Minimum |
| Healthy | 21 | 56.8 (±30.2) | 97.1 | 7.0 |
| Brown-stain | 21 | 29.7 (±30.2) | 93.0 | 0.8 |
| Resinosis | 17 | 9.9 (±8.2) | 29.8 | 0.0 |
| Black-stain | 12 | 1.7 (±3.2) | 13.7 | 0.0 |
| Other | 4 | 1.9 (±7.2) | 32.8 | 0.0 |

[a] Based on 21 of the 23 diseased trees. Not included was the tree that died prior to harvest, and another tree where rot had destroyed the taproot making an estimate of the affected area unreliable. Root diseases are the same as reported in Kelsey et al. (1998); the brown-stain is probably from *Heterobasidion annosum*; black-stain is *Leptographium wageneri* var. *ponderosum*, the other category includes blue-stain from *Ophiostoma* spp. Both *H. annosum* and *L. wageneri*, as well as other root pathogens, can cause resinosis without stain. Root systems of all 21 trees had some healthy tissue, resinosis (except four), and at least one pathogen. Seven trees had resinosis plus two pathogens, and four had resinosis plus three pathogens.

trees in crown classes 1 and 3 (Fig. 2), except that the magnitudes of the differences between the diseased and healthy categories were smaller. There were no differences in age, diameter, or height between the diseased and healthy trees.

Acetaldehyde and acetone concentrations were greater in class 1 trees than those in classes 2 and 3, with no differences in methanol or ethanol concentrations among any of the three crown classes (Fig. 4). Sapwood water content was lowest in class 1 and highest in class 3. When trees were categorized by their root disease status, those with disease contained higher quantities of all four chemical constituents, and lower sapwood water contents than those without disease (Fig. 5).

One of the diseased trees died during the interval between initial selection and root extraction and was not included in the chemical data set analyzed statistically, but was included in the data set used to evaluate performance of the logistic regression models. This tree had the highest acetaldehyde (2.60 μmol/g dry wt.) and ethanol (7.49 μmol/g dry wt.) concentrations, and the 7th lowest sapwood water content (85.58% dry wt. basis) of all the trees analyzed.

Of the 32 logistic regression models compared using dry weight compound concentrations and sapwood water content, the first 16 models at the top of the list had ln(acetone) as an explanatory variable (Table 2). The value of ln(acetone) as an explanatory variable is shown by summing the Akaike weights for all models containing this variable, which yields 0.9732. This is about double the summed Akaike weights for all models with ln(acetaldehyde) 0.4323, ln(methanol) 0.4691, ln(ethanol + 0.05) 0.4542, or sapwood water content, 0.5089. Although model 1D had the lowest $AIC_c$ (Table 2), and was selected the best among those with dry weight

$$\text{prob. of root disease} = \exp^{(4.962+((1.983\times\ln\text{ acetone})-(-0.023\times\%\text{water})))}/(1 + \exp^{(4.962+((1.983\times\ln\text{ acetone})-(-0.023\times\%\text{water})))}) \quad (1D)$$

concentrations for predicting trees with root disease, it does not stand out as an appreciably better model than the next five lower ranking models, 2D–6D, because its $AIC_c$ and $w_i$ values were not substantially different from theirs (Table 2). Performance tests with the current data set indicated models 1D–6D performed similarly (data not shown), in which case the principal of parsimony suggests model 6D, with the fewest parameters, might be preferred (Burnham and Anderson, 1998). Model 6D performed nearly the same as 1D when cut-off

$$\text{prob. of root disease} = \exp^{(2.783+(2.382\times\ln\text{ acetone}))}/(1 + \exp^{(2.783+(2.382\times\ln\text{ acetone}))}) \quad (6D)$$

probabilities of 0.25 or 0.35 were used for identifying healthy or diseased trees (Table 3). At the 0.45 cut-off values model 6D was less effective than model 1D at correctly identifying diseased trees.

We also compared models using fresh weight concentrations for all compounds rather than the dry weight concentrations discussed above. Here again the top 16 models all contained ln(acetone), but the best model (1F) contained only ln(acetone) as an explanatory variable with an Akaike weight of 0.1417:

$$\text{prob. of root disease} = \exp^{(3.769+(2.054\times\ln\text{ acetone fresh wt.}))}/(1 + \exp^{(3.769+(2.054\times\ln\text{ acetone fresh wt.}))}) \quad (1F)$$

Model 1F, like 1D, did not stand out from the next six lower models on the list, but since it had the fewest explanatory variables it was considered the best from this group. The performance of model 1F was comparable to models 1D and 6D (Table 3).

Models 1D and 1F at cut-off values of 0.25, 0.35, and 0.45 identified the 10 diseased trees with the greatest % non-functional root area (ranging from 42.4 to 93.0%, mean of 72.2%). Model 6D also identified these trees, except at cut-off level 0.45. Trees with lower levels of disease (ranging from 2.9 to 41.4%, mean 21.4% non-functional root area) did not exhibit sufficient changes in chemical concentrations to be recognized as diseased by most models.

Within the group of 23 trees with root disease, four of the headspace compounds showed positive relationships with the % non-functional root area at about 38 cm from the root collar

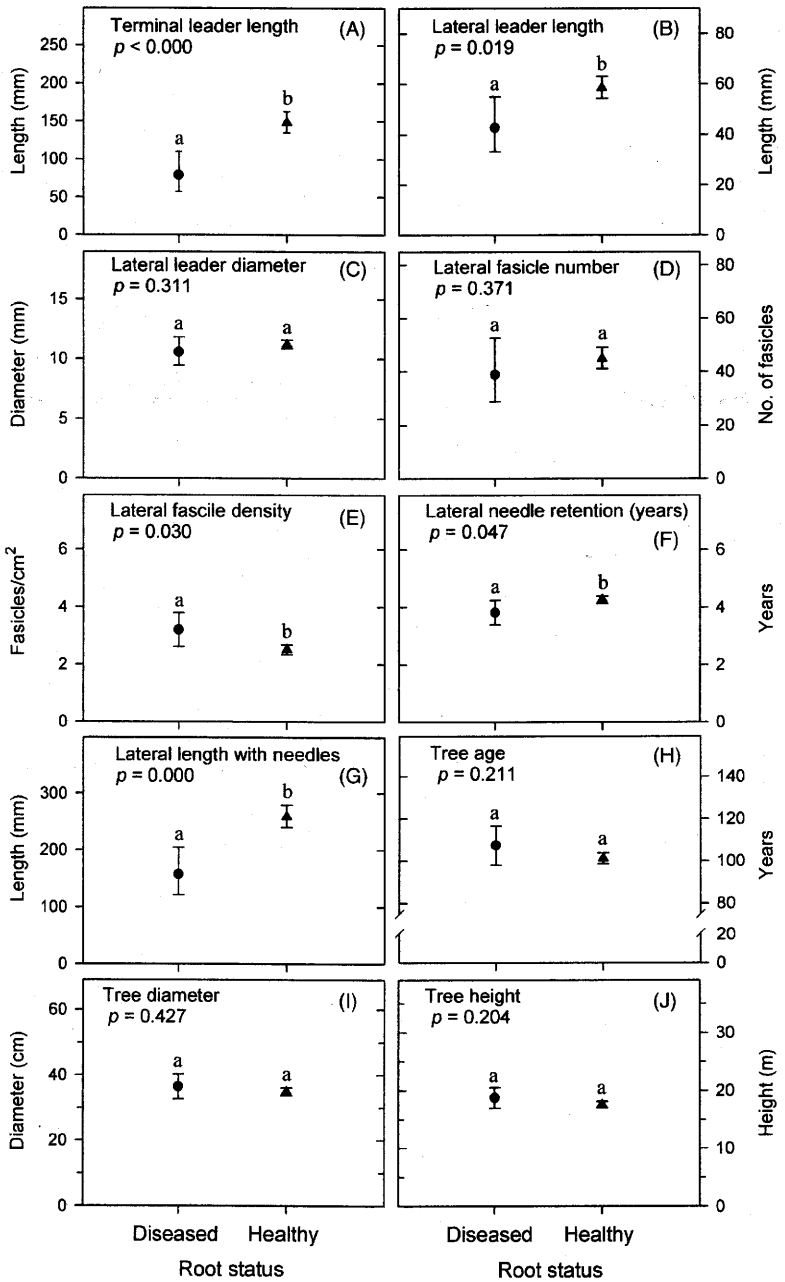


Fig. 3. Foliar and stem parameters measured for trees with and without root disease. Graphs A, B, D, G show medians ± 95% CI, and all others show means ± 95% CI. $p$ Values are from the GLM analysis. Symbols with the same letters above are not significantly different at $p = 0.05$.

(Fig. 6). Natural log transformed acetaldehyde and acetone concentrations were strongly related with the non-functional root area, while methanol and ethanol were more moderately related. The sapwood water content had a strong, negative relationship with the % non-functional root area.

## 4. Discussion

Crown class, or the visual severity of a lion's tail appearance for branches in the upper portion of the crown, was not a reliable symptom of root disease at this study site. While eight of the 21 trees in crown class 1 were diseased (38.1%), there were also eight diseased trees in crown class 2, and seven in class 3. Of 115 trees with lion's tail appearance, 99 did not have root disease. Thies et al. (2005) examined sapwood near the root collar for evidence of black-stain root disease in ponderosa pine killed during spring and fall prescribed burns at nearby study sites (Fig. 1). They concluded the lion's tail appearance of branches was not a reliable symptom for this disease.

The expression of differences in crown morphology for trees in crown classes 2 and 3 probably results from the combined effects of tree size and levels of resources available on site to support growth. Trees in crown class 3 were shorter, younger, and growing more rapidly than those in class 2, thus making

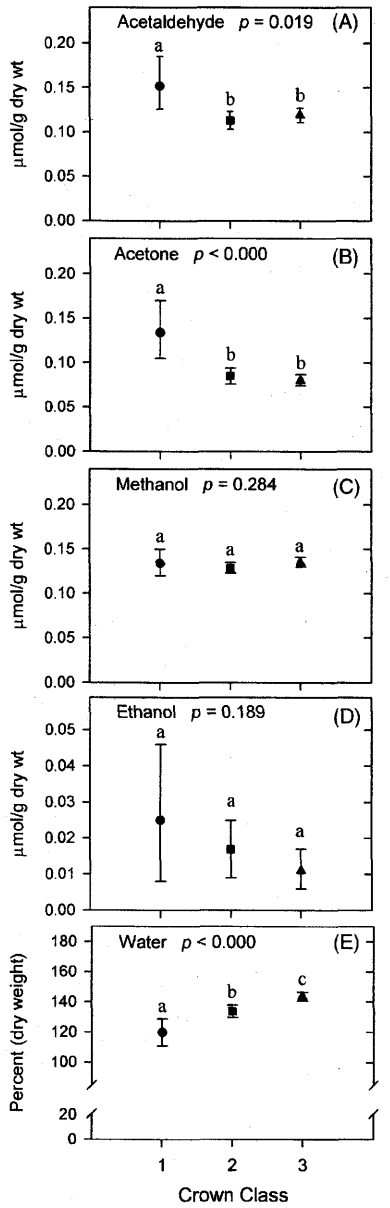


Fig. 4. Median (±95% CI) concentrations of headspace compounds, and mean (±95% CI) water content for sapwood collected near the root collar of trees in each of the three crown classes. $p$ Values are from the GLM analysis. Symbols with the same letters above are not significantly different at $p = 0.05$.

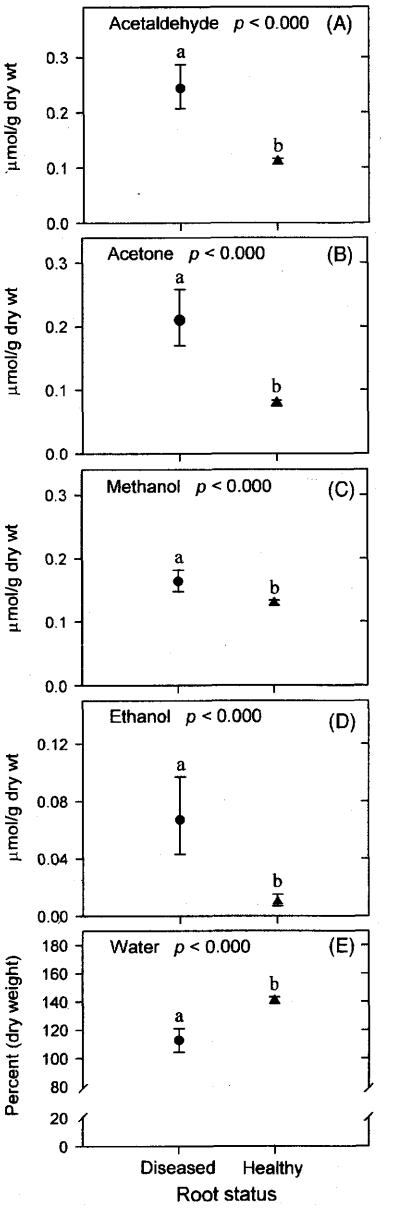


Fig. 5. Median (±95% CI) concentrations of headspace compounds, and mean (±95% CI) water content for sapwood collected near the root collar of trees with and without root disease. $p$ Values are from the GLM analysis. Symbols with the same letters above are not significantly different at $p = 0.05$.

them appear more vigorous and healthy looking. As shown recently for Scots pine, *Pinus sylvestris* L., and three hardwood species, tree size mediates vigor or growth (Mencuccini et al., 2005), as do the availability of resources, especially water and nutrients (Ryan and Yoder, 1997). Water is a critical factor at our site near the southern end of the Blue Mountains with average annual rainfall ranging from 38 to 51 cm, and just a few kilometers north of the forests edge (Franklin and Dyrness, 1988), where limits on water availability probably contributes to the transition into shrub-steppe. The sapwood water contents of class 2 trees were lower than the class 3 trees. Thus, we speculate that the vigorous, healthy looking trees in class 3 begin expressing crown characteristics of class 2 when they reach a size that can no longer sustain vigorous crown growth, because of limited water availability. Drought stress in red pine reduces shoot length growth and increases fascicle density (Clements, 1970) compared to trees without water deficits, causing changes in crown growth similar to those observed between the classes 2 and 3 trees.

Trees in crown class 1 had the lowest sapwood water contents (Fig. 4E), and the shortest length growth of terminal leaders and lateral branches (Fig. 2A and B) indicating they were probably exposed to a greater intensity of stress than trees in class 2. Eight (38.1%) of the 21 class 1 trees were stressed by root disease, which can negatively impact water uptake or flow (Hessburg, 1984; Hessburg and Hansen, 1987; Joseph et al.,

Table 2
The top 16 of 32 logistic regression models (with sapwood dry weight compound concentrations and water content) for predicting the probability of an individual tree being infected with root disease pathogens

| Model no. | Model explanatory variables | $K$ | MLL | $AIC_c$ | $\Delta_i$ | $w_i$ | Cum $w_i$ |
|---|---|---|---|---|---|---|---|
| 1D | Ln(Ace), $H_2O$ | 3 | −53.939 | 113.963 | 0.0000 | 0.1114 | 0.1114 |
| 2D | Ln(Ace), Ln(EtOH + 0.05) | 3 | −54.141 | 114.369 | 0.4052 | 0.0909 | 0.2023 |
| 3D | Ln(ETO), Ln(Ace), Ln(MeOH) | 4 | −53.232 | 114.608 | 0.6443 | 0.0807 | 0.2830 |
| 4D | Ln(Ace), Ln(EtOH + 0.05), $H_2O$ | 4 | −53.321 | 114.786 | 0.8223 | 0.0738 | 0.3568 |
| 5D | Ln(ETO), Ln(Ace), Ln(MeOH), $H_2O$ | 5 | −52.377 | 114.970 | 1.0062 | 0.0673 | 0.4241 |
| 6D | Ln(Ace) | 2 | −55.473 | 114.989 | 1.0252 | 0.0667 | 0.4908 |
| 7D | Ln(Ace), Ln(MeOH), $H_2O$ | 4 | −53.438 | 115.020 | 1.0566 | 0.0657 | 0.5564 |
| 8D | Ln(Ace), Ln(MeOH), Ln(EtOH + 0.05) | 4 | −53.464 | 115.071 | 1.1077 | 0.0640 | 0.6204 |
| 9D | Ln(Ace), Ln(MeOH), Ln(EtOH + 0.05), $H_2O$ | 5 | −52.478 | 115.172 | 1.2088 | 0.0608 | 0.6813 |
| 10D | Ln(ETO), Ln(Ace) | 3 | −54.645 | 115.376 | 1.4126 | 0.0550 | 0.7362 |
| 11D | Ln(ETO), Ln(Ace), Ln(MeOH), Ln(EtOH + 0.05) | 5 | −52.591 | 115.398 | 1.4342 | 0.0544 | 0.7906 |
| 12D | Ln(ETO), Ln(Ace), $H_2O$ | 4 | −53.702 | 115.547 | 1.5838 | 0.0504 | 0.8410 |
| 13D | Ln(ETO), Ln(Ace), Ln(EtOH + 0.05) | 4 | −53.984 | 116.111 | 2.1476 | 0.0381 | 0.8791 |
| 14D | Ln(ETO), Ln(Ace), Ln(MeOH), Ln(EtOH + 0.05), $H_2O$ | 6 | −51.943 | 116.190 | 2.2262 | 0.0366 | 0.9156 |
| 15D | Ln(Ace), Ln(MeOH) | 3 | −55.232 | 116.551 | 2.5874 | 0.0305 | 0.9462 |
| 16D | Ln(ETO), Ln(Ace), Ln(EtOH + 0.05), $H_2O$ | 5 | −53.289 | 116.795 | 2.8318 | 0.0270 | 0.9732 |

The models were ranked in a simultaneous comparison using the information-theoretic model selection method (Burnham and Anderson, 1998). *Note*: $K$, number of estimable parameters in the model; MLL, maximum log likelihood; $AIC_c$, Akaike's information criterion with bias adjustment for small samples; $\Delta_i$, $AIC_c$ difference relative to the smallest $AIC_c$ value in the set; $w_i$, Akaike weights, estimates of the likelihood of the model, given the data; Cum $w_i$, cumulative Akaike weights. Ln = natural logarithm; Ace = acetone, ETO = acetaldehyde, MeOH = methanol, EtOH = ethanol concentrations.

1998) and account for their lower sapwood water content and reduce crown growth compared to healthy trees (Fig. 4). Although the 13 remaining class 1 trees were disease free, they still may have been stressed by water deficits resulting from growth on micro-sites with shallow, dry soils, or they were located in an area with higher tree densities, where competition for water was greater than typically occurs elsewhere.

Ponderosa pine with root disease contained higher dry weight concentrations of all the sapwood headspace compounds, and lower sapwood water contents than trees with healthy roots (Fig. 5). Within the group of diseased trees, sapwood water content had the strongest relationship with non-functional root area, and also the only negative relationship (Fig. 6E). Relationships of acetaldehyde and acetone concentrations with the % non-functional root area were of similar strength (Fig. 6A and B), but in this study acetone, rather than acetaldehyde (Kelsey et al., 1998), was the most useful compound for identifying diseased trees. It is interesting that the tree with the highest June ethanol concentration, did not survive the summer. Abnormally high sapwood ethanol concentrations near the root collar are strong indicators of disease in the root system (Kelsey and Joseph, 1998; Kelsey et al., 1998), provided the trees have not been recently flooded (Joseph and Kelsey, 1997), or injured by fire (Kelsey and Joseph, 2003). But, low ethanol concentrations are not reliable indicators the roots are healthy, and within the group of diseased trees it did not have a strong relationship with non-functional root area here (Fig. 6D), or in the earlier study (Kelsey et al., 1998).

Table 3
Partial comparison of performance (at three cut-off values) for three logistic regression models selected as best for predicting the probability of an individual tree being infected with root disease pathogens

| Model no. | Model explanatory variables | Cut-off prob. for sorting[a] | % Identified correctly | | |
|---|---|---|---|---|---|
| | | | Diseased | Healthy | Total |
| 1D | Ln(Ace), $H_2O$ | 0.25 | 43.5 | 98.5 | 94.0 |
| 6D | Ln(Ace) | 0.25 | 47.8 | 98.1 | 94.0 |
| 1F | Ln(Ace)—fresh wt. conc. | 0.25 | 43.5 | 98.5 | 94.0 |
| 1D | Ln(Ace), $H_2O$ | 0.35 | 43.5 | 99.2 | 94.7 |
| 6D | Ln(Ace) | 0.35 | 43.5 | 98.9 | 94.4 |
| 1F | Ln(Ace)—fresh wt. conc. | 0.35 | 43.5 | 98.9 | 94.4 |
| 1D | Ln(Ace), $H_2O$ | 0.45 | 43.5 | 99.2 | 94.7 |
| 6D | Ln(Ace) | 0.45 | 30.4 | 99.2 | 93.7 |
| 1F | Ln(Ace)—fresh wt. conc. | 0.45 | 43.5 | 99.2 | 94.7 |

Acetone concentrations were calculated on a dry weight basis in models 1D and 6D and fresh weight basis in model 1F. Performance of each model is evaluated by the percentage of trees in the current data set they predicted as disease or healthy, in comparison to the trees observed root disease status.

[a] The cut-off probability used for comparison with the model calculated probability for each tree in the data set. When the model calculated probability was $\geq$ the cut-off probability the tree was identified as diseased, otherwise it was considered healthy. Model predictions were compared with the observed root disease status of each tree to determine the % identified correctly.

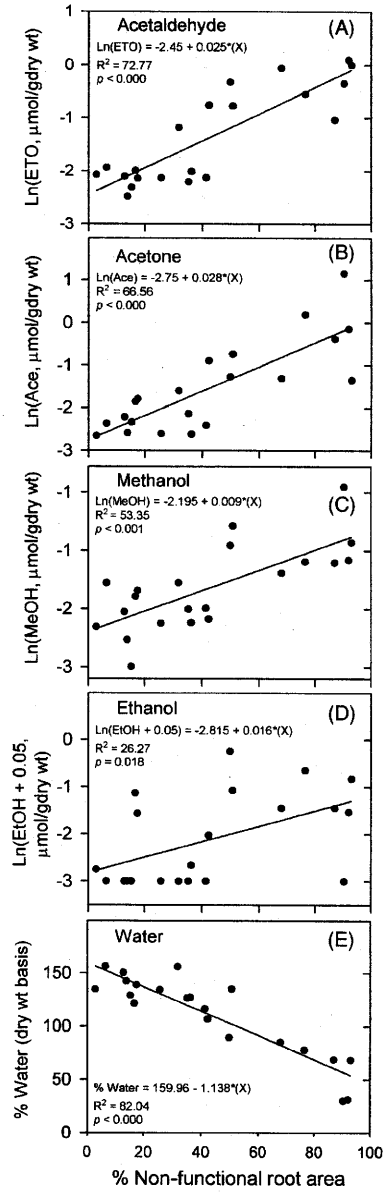


Fig. 6. Relationships between the headspace compounds or water content from sapwood collected near the root collar with the % of the total root system cross-sectional area that was non-functional at 38 cm below the root collar, for trees with root disease. Based on 21 of the 23 diseased trees, see Table 1 note.

Models 1D, 6D, and 1F, in nearly every instance identified the 10 trees with the highest non-functional root area (mean 72.2%). In contrast, none of these models identified diseased trees with 40.0% or less non-functional root areas. Diseased trees misidentified by the models had a mean non-functional root area of 21.4%. But, trees with this degree of reduction in root function would not be expected to show crown symptoms either, as this is well below the level of mechanical root removal needed to cause measurable symptoms of impaired water supply in the crown of subalpine fir (Teskey et al., 1985), and the level of root disease considered necessary for crown symptoms to develop in ponderosa pine (Kelsey et al., 1998). Furthermore, at this site the expression of crown symptoms in response to root disease was being confounded by limitations on crown growth resulting from water deficits associated with the site and other stresses.

Although the three models were similar in their performance for identifying diseased trees there are practical reasons for preferring model 1F. First, acetone concentrations could be calculated from a single GC analysis of each sample. For this experiment we used a multiple headspace GC technique that allows accurate concentrations of ethanol to be calculated in the tissues, but this procedure requires the sample be analyzed twice by GC. Unlike ethanol, acetone is generated from the tissue during the heating of the sample (Kelsey et al., 1998) and is estimated from the first GC analysis. Eliminating the second analysis would double the rate of sample processing. Second, calculating concentrations on a fresh weight basis would eliminate the need to dry samples following GC analysis, further increasing the process rate. Thus, we select model 1F as the best for predicting root disease in ponderosa pine at our study site because it is the simplest and easiest to use among the final three being compared. Also, the sapwood analysis rate in the lab could be increased four times by combining the four samples of sapwood from each tree into one vial rather than analyzing them each separately. With this modification acetone fresh weight concentrations could be measured on approximately 1000–1500 trees during a 5-day period. This would make sample collection the limiting step, at a rate of about 80 ± 20 trees/day for a two person field crew. This rate could change depending on variables such as spacing between sample trees, terrain, and weather.

Acetone fresh weight concentrations could be useful for identifying diseased trees for removal from the stand, or in more general surveys for estimating levels of disease. As an example, assume the 284 trees we sampled in this experiment represent the entire population in a high value area. Using model 1F with a cut-off probability of 0.45 for sorting healthy and diseased tree, we would select the 10 most severely diseased trees (43.5% of all diseased trees), and two healthy trees for removal. An alternative approach would be to remove the 21 trees in crown class 1. While, this would eliminate 8 diseased trees, they include only 4 of the 10 most severely diseased, plus 13 trees without disease, or 6.5 times the number of healthy trees removed using model 1F. Another approach is to remove all trees in crown class 1, plus those identified by model 1F. This would eliminate 14 or 60.9% of all the diseased trees, including the 10 most severely diseased, plus 15 healthy ones, leaving 93.3% of the initial population. Although this seems acceptable, it requires removing 2.4 times more trees than model 1F alone, in exchange for a small increase in the proportion of diseased trees eliminated (from 43.5 to 60.9%). Model 1F appears better at selecting diseased trees for removal than using crown classes, or the two methods in combination.

With random sampling, acetone fresh weight concentrations might also be useful for estimating the total number or proportion of diseased trees within a stand. Although model 1F does not identify trees with less than 40% non-functional root area, the total number of diseased trees might be estimated as falling between two and three times the number of individuals

with severe disease. Model 1F identified 10 severely diseased trees, and would have estimated 20–30 diseased trees total, for an actual population of 23 detected by root excavation. In stands like the ones in this study, where crown symptoms are unreliable indicators of root disease, this estimate may be as accurate as can be obtained without excavating the roots.

When sampling diseased trees the likelihood of spreading the pathogen to healthy trees is always a concern, but this can be minimized by disinfecting the increment bit, or similar sampling tool, between trees. This precaution was not necessary for this study since all the trees sampled were harvested.

In conclusion, acetone fresh weight concentrations in headspace from sapwood of ponderosa pine collected near the root collar shows promise for monitoring annosum and black-stain root disease at dry-sites in eastern Oregon. However, it still requires further study with greater numbers of diseased trees over a larger geographic area, representing a broader range of environmental conditions and disease conditions, including other pathogens such as *A. ostoyae*.

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