



## AN ABSTRACT OF THE THESIS OF

Alexandra M. Peterson for the degree of Honors Baccalaureate of Science in Environmental Science presented on May 30, 2014. Title: Specific Growth Rings to Which Douglas-fir Needles Are Attached: Site Variability

Abstract Approved: \_\_\_\_\_  
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Stands of Douglas-fir (*Pseudotsuga menziesii* ssp. *menziesii* Mirb. (Franco)) near the Oregon Coast are showing signs of growth reduction from the foliar pathogen *Phaeocryptopus gaeumannii* that causes Swiss Needle Cast (SNC) disease. To better understand the mechanisms of needle drop through disease, and to learn the degree of variability in needle attachment, I tested the extent to which 3-year old needles have connections to xylem produced in the two years after needles were formed. I submerged the base of a stem with one needle attached into a basin of stain, and then placed the needle, whose tip had been cut off, into a vacuum for 5 minutes. I then surfaced the stem with a razor blade, to learn into which rings the dye had entered when sucked from the needle. Contrary to expectations, there was no significant difference in the proportion of 2011 needles that drew stain through only xylem produced in 2011. However, also in contrast to expectations, two of the three high-disease-impact sites had a higher number of connections than the three low-disease-impact sites, suggesting that there may be a disease effect. There was a wide variety of needle attachment patterns at both the site and region levels, which is important for physiologists when they extrapolate tree productivity from sap flow research.

Key Words: needle morphology, Douglas-fir, hydraulic architecture, Swiss needle cast

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Specific Growth Rings to Which Douglas-fir Needles Are Attached:

Site Variability

by

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Alexandra M. Peterson

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## Specific Growth Rings to Which Douglas-fir Needles Are Attached: Site Variability

### Introduction

Swiss Needle Cast (SNC) is a foliage disease caused by the fungal pathogen *Phaeocryptopus gaeumannii* and is unique to the genus *Pseudotsuga* and common throughout Douglas-fir. The fungus itself is native to the endemic range of Douglas-fir in North America consisting largely of British Columbia, Washington, and Oregon. Due to the effects of disease Douglas-fir trees that are heavily affected by SNC exhibit high rates of needle abscission. A healthy Douglas-fir tree typically retains about five needle cohorts on average (Stone et al. 2007), whereas severely diseased individuals typically retain less than three cohort years and their ability to effectively control gas exchange is severely inhibited. Growth decline is another major issue within diseased stands and can have serious implications in the commercial value of these trees. A monetary loss within the timber industry is another risk, as well as potential detrimental effects on wildlife species that are dependent on Douglas-fir. Plantations of Douglas-fir within parts of Europe, Chile, Australia, and New Zealand are also experiencing the plight of SNC on local timber industries (Kimberley et al. 2010), indicating the global scale to which this disease is relevant. Extensive research efforts have been undertaken since the 1990 epidemic of SNC, largely as a result of the changes in disease severity witnessed in the Pacific Northwest. While disease is one aspect of declining growth and photosynthetic rates in Pacific Northwest stands, sapwood flux is also a typical measure, or proxy, for tree productivity (i.e. vigor) and might be influenced by variability in needle attachment.

The purpose of this study is to explore the effects of foliage disease on the pattern of needle attachment in Douglas-fir (using needle retention as an index of disease severity) and to explore the variability of attachment patterns by site and region.

### Needle Attachment

When a new needle is formed at the cambium, it makes a structure called the trace, which has one part that interfaces physically with the branch's xylem and another part that interfaces with the branch's phloem. After a period of xylem growth, the trace can still be seen, extending from the petiole inward horizontally and into the xylem. The needle trace connects the needle's xylem to the branch's xylem in the growth ring from the same year it was made. As new wood is added in subsequent years, there's an opportunity for the needle trace to connect to new growth rings. It's possible that the old connection to the xylem from the year the needle was produced will break, from either the stretching or breaking of tracheids as the needle trace extends to newer growth rings (Elliott, 1937) (Fig.1). It is unknown what determines whether a needle trace will break or form new functional connections to xylem produced in later years. Functional connections to the xylem aid in water transport, because water is being sucked upward through the xylem and out by the foliage where it is lost during photosynthesis. Therefore, sapwood flow might be dependent on the spatial distribution and variability of these functional needle connections. It could be that having multiple active connections is advantageous for more readily accessing water and thus have a greater sap flux based on distribution of these connections.

Previous studies have examined leaf trace anatomy in conifers (Tison 1903; Elliott 1937) and have focused on needle attachment variability across multiple species (Maton and Gartner 2005). Maton and Gartner (2005) found that at very large stem diameters, only found on the leader, there was a positive correlation between diameter and needle abscission in Douglas-fir. It might be possible that a disease such as Swiss Needle Cast, which is known to decrease growth rates (Hansen et al. 2000), might end up promoting longer needle connection longevity. It is possible that a three-year old needle could have the following combination of connections to growth rings: 2011 (the original), 2012, 2013, 2011+2012+2013, 2011+2012, 2012+2013, or 2011+2013. We refer to the variability as the state of having connections different from the simple original (2011) connection. Variability in needle attachment can refer to any pattern that is different than the original connection. Not all Douglas-fir (*Pseudotsuga menziesii* ssp. *menziesii* Mirb. (Franco) needles form new connections, and even those that do, don't always lose their old connections. This study examined 3-year old Douglas-fir needles across a range of disease severity in Oregon, and found that a single needle could be attached to all three growth rings (forming a new connection every year after its formation) or it might only retain its original connection without making new connections to the younger xylem. This variability could be important in bettering our understanding of water transport within a tree.

I chose to look at needle attachment patterns in trees growing in two distinct regions, near the coast (Coastal), and on the eastern slope of the Coast Range (Coast Range). The Coastal region is known to have relatively higher impact of SNC than the Coast Range region (Maguire et al. 2011), and so the study was designed to interpret the

regional comparison as a high vs low disease impact comparison. I acknowledge that other factors besides disease severity differ between the regions, and my inferences are made with this caveat in mind.

## History

The historical range of *P. gaeumannii* is important to understand in the context of this problem. The earliest record of the fungus occurred in 1915 near Roseburg, Oregon when it was unintentionally collected from trees heavily infected with *Rhabdocline*, a fungal pathogen which causes symptoms similar to SNC (Boyce 1940). Although it was prevalent in the area, there were no signs that *P. gaeumannii* was contributing to premature needle casting (Boyce 1940). Although the fungus was recognized in the Pacific Northwest nearly a decade earlier, the disease gets its name from the first documented case which occurred on a plantation in Switzerland in 1925 (Boyce 1940). Historically, stands of severely infected Douglas-fir in the Pacific Northwest have occurred within approximately 30km of the coast, with high infection happening in the “fog belt” at lower elevations. However, recent trends point to possible inland expansion. It’s thought that this recent outbreak of SNC might be due to local changes in climate which are expanding the range of favorable conditions for *P. gaeumannii* infection and development. Past research has found that warmer winters and wetter springs and summers have provided such favorable conditions, and that expected trends in climate change for the Pacific Northwest (about a 0.4°C increase per decade) suggest SNC will become more abundant and more severe in the future (Stone et al. 2008).

## Effects of SNC

Symptoms of SNC include decreased gas exchange between the tree and its environment; as a result, chlorotic needles are a common sight among infected stands of Douglas-fir. This is due to the blockage of stomata on the underside of needles by the ascocarps of *P. gaeumannii*, which are technically referred to as pseudothecia. A severely diseased individual will show numerous pseudothecia on its needles and as a result will prematurely cast those needles (i.e. have heavy rates of needle abscission). Needle abscission typically occurs when the needle reaches a threshold and becomes a carbon sink (Manter et al. 2003). Heavily diseased trees have sparse crowns (Weiskittel and Maguire 2007) and only retain one-to-two years of needles (Hansen et al. 2000), all of which can influence net canopy photosynthesis. Blocked stomata also have implications in the control of water transport between the various parts of the tree by affecting relative transpiration rates. If an individual is experiencing high incidence of disease (as measured by number of needles with at least one pseudothecia) and increasing proportions of blocked stomata, photosynthesis and transpiration rates are affected and result in severely reduced rates of carbon assimilation (Manter et al. 2003). The challenges imposed by SNC might also lead to diversion of energy within an individual. Previous studies have found that storage of available carbohydrates is prioritized over growth in trees with SNC (Saffell et al. 2014). These studies do not take into account patterns of needle attachment, and how this might affect prioritization of available photosynthates for either storage or growth. It could be possible, that sequestering carbohydrates (instead of investing in production and growth) might be tied to some mechanism that also prioritizes the formation of new connections between needles and

wood produced in subsequent years. This study found that diseased trees typically tend to exhibit higher rates of multiple needle connections between infected needles and xylem produced in years after original needle formation.

This disease influences productivity, namely by decreasing the overall photosynthetic capacity of the individual which produces declines in growth rates but rarely results in early tree mortality even within stands of high disease severity. Suppressed growth rates can have huge ramifications within local timber industries. For example, Douglas-fir is one of the most common and abundant species used in the Pacific Northwest for Christmas tree plantations. It was found that a monetary loss of \$3.4 million dollars was experienced in 1981, directly attributable to needle loss among Douglas-fir Christmas tree plantations in Western Oregon and Washington (Michaels and Chastagner 1984). Postharvest quality is also reduced by decreased needle retention in commercially grown Douglas-fir. Not only is SNC attributed with declines in needle retention, growth rate (Hansen et al. 2000), and wood density (Johnson et al. 2003), but it is also associated with a decrease in incidence and diversity of favorable symbiotic relationships to mycorrhizal fungi (Luoma and Eberhardt 2014). Many of these factors weaken the tree and can cause it to lose any competitive advantage over healthier individuals or other species.

Douglas-fir provides the matrix for habitat for a very large proportion of the animal species found in the Coast Range. With reductions in productivity and crown density, many species could be negatively affected by this change in cover and/or food availability. Increased predation could be a result of reduction in cover within the canopy

for many bird species that use specific places in the canopy for nesting or cover. More research should be done to evaluate the effects of SNC on wildlife species.

### Indices of Disease Severity

To determine the degree to which an individual tree is affected by SNC there are several methods of measurement available. Needle retention is a qualitative approach to assessing the degree of severity within an individual based on the number of needle cohorts remaining on an infected tree (measured in years). Typically, severely diseased trees retain fewer than three years of needles. In this study, four year old lateral branches were examined to test the pattern of needle attachment from several sites of high and low SNC severity. These branches were assessed on a scale of 0-4.0 based on the maximum number of needle cohorts per sample. For example, a sample displaying only one- and two-year-old needles would be given a needle retention value of 2.0 indicating moderately severe infection. Along with needle retention, pseudothecial counts are also frequently used to establish severity on the individual level, as well as disease incidence. This involves estimating the proportion of open to blocked stomata on the underside of infected needles (where level of incidence is recorded as total number of needles with at least one pseudothecia present). These counts were beyond the scope of the project but were undertaken in a companion study. Diameter can be used as a way of measuring differences in growth between healthy and infected trees, as decreased growth rates in both diameter and height are expected for trees with average needle retention less than 3.0-3.5 years (Mulvey et al. 2013).



## Objective

The objective of this study was to i) learn the degree of variability in needle attachment patterns by region and site in Douglas-fir, and ii) to shed light on the mechanisms of needle drop with SNC disease. The hypothesis is that trees with relatively higher SNC impact will exhibit fewer needle connections than trees with lower SNC impact. The rationale for this hypothesis is physiological: if the higher-disease-impacted trees have limited productivity, then at the tree level, the resources available might be prioritized to the younger needles which have higher photosynthetic rates (Meinzer 1982). Younger needles are attached, by developmental rules, to the younger wood. If old needles do not attach to the younger wood, they may not compete as directly with the younger, more productive needles for resources.

## Methods

### Field Site and Sampling:

A series of growth impact studies throughout the SNC impacted area of western Oregon (Maguire et al. 2011) gave a basis for choosing sites where tree growth has been heavily vs. slightly impacted by SNC in recent years (called Coastal and Coast Range, respectively). I sought planted forests of similar age (planted in 2004 if possible) within both the Coast Range and Coastal regions then chose sites based on availability and access. The three high SNC sites within the Coastal region were offered by Plum Creek Timber (hereafter referred to as PCA, PCB, and PCC) and all occurred within 10 km of the Pacific Ocean (Table 1). The three low SNC sites within the Coast Range region are Good Neighbor, Wapiti, and Yellow Jacket (on the McDonald-Dunn Research Forests, Oregon State University, hereafter referred to as MA, MB, and MC, respectively) all on the eastern slope of the Oregon Coast Range, about 60 km from the Pacific Ocean (Table 1).

Trees were sampled in January-April 2014, before the 2014 needles had emerged from the buds. Thirty trees were sampled from the first high SNC and the first low SNC sites (PCA and MA, respectively) but I decreased sample size to 15 trees/site thereafter on the basis of the variability at the first sites and due to time constraints. I sampled easily accessible trees that met these criteria: they were not immediate neighbors, they were not unduly deformed (e.g., forked or had broken tops), and they were not much smaller than other trees in the stand as assessed subjectively. I removed one branch from each tree for analysis in the lab, using the standard branch assessed by the Swiss Needle

Cast Cooperative for needle retention (Hansen et al. 2000, Kanaskie and Maguire 2002). The branch was from the fifth whorl down from the top (thus, aged 5 at its base), and from that branch I removed all but the bottom pair of branchlets (thus, each was aged 4 at their bases), leaving them connected to their parent branch (Fig. 3). All branches from a site were kept in the same dark plastic bag that also contained a wet paper towel, and were transported rapidly to the lab. There, the base of each stem was put into a water-filled bucket, and the entire assemblage was again covered with a dark plastic bag and placed in a cold room (3 °C) until analysis, usually within 7 days of harvest. One of the two branches was then used for all experiments; the second was retained as a back-up in case the first sample had an unforeseen defect or subjected to experimental error.

#### Laboratory analyses:

One of the two branchlets was randomly selected for further analysis. Needle retention for that four-year old branchlet was recorded as an estimate of the foliage longevity. A sample with all of its 1- and 2-year old needles but no 3-year old needles would have a needle retention value of 2.0 years. In contrast, if the sample also had about 70% of its 3-year old needles, it was given a value of needle retention of 2.7. This value is determined subjectively (following Kanaskie and Maguire 2002).

I then assessed to which growth rings 3-year old needles (and 2-year old needles for the first two sites) drew water. First, I chose a two centimeter long segment of stem of either 3 (all sites) or 2 (first two sites) years old, and separated it from the rest of the sample with hand clippers. I then removed all but one needle (located toward the distal

end) from the branch. The segment was then placed in a water-filled tray where I re-trimmed the basal part of the branch with a fresh razor blade, to create a surface in which the cross-sections of all of the tracheids were exposed and to remove any compressed or sheared material left by the clippers. The distal 5mm of the needle was then removed with a razor blade and inserted into a slit in the sidewall of latex tubing such that the exposed tip was inside the tube (Fig. 4). The tube was attached to the building's vacuum system on one end and closed off on the other end. The base of the branch segment was submerged in a shallow reservoir filled with 1% (weight/volume) aqueous acid fuchsin stain (Maton & Gartner 2005). The reservoir was fabricated from a 1.5 cm (inner diameter) piece of tubing that was cut to 1 cm in length and closed at its base with Parafilm™.

The sample was subjected to the vacuum for 5 minutes, during which time the pull on the needle's tip simulated the pull from transpiration. Any liquid pulled upward through the needle should have been replaced by liquid in the reservoir. The sample was then removed from the basin of stain. At its base, the sample was entirely colored with stain, but after I trimmed off a small amount with a razor blade (usually 3-6 mm) the stain was confined to distinct spots. I examined the trimmed base under a dissecting microscope at 10-60x magnification to assess in which growth ring the stain was located. For the 3-year old needles, which were produced in 2011, I recorded the growth rings containing stains as those produced in 2011, 2012, or 2013. For the 2-year old needles, which were produced in 2012, I recorded the growth rings containing stains as those produced in 2012, or 2013.

I archived the samples by gluing their transverse ends onto labelled locations on sheets of paper so that the surface viewed under the microscope was facing up. Once samples had dried, I measured their diameter under bark using digital calipers, for use as a possible explanatory variable for pattern of needle attachment.

#### Statistical analyses:

An exploratory analysis of data was first done to examine differences in the means and variances between regions and sites. Means plots were used to graphically show differences between sites sampled. This was done for each explanatory variable (needle retention and diameter) and for average proportion of staining patterns that occurred in only the 2011 growth ring across sites. Pooled averages of sites within regions were used to construct means plots for each variable between regions.

An analysis of variance (ANOVA) was used to distinguish differences between the mean values of needle retention (measured in years) between regions. However, plots of residuals vs. predicted values violated the assumption of equal variance. To account for this, the data were transformed using a logit transformation with PROC GLIMMIX in SAS. This analysis also found that a gamma distribution model was the best fit for the data. This was seen by examining the fit statistics, comparing a poisson distribution and gamma distribution. After the data were transformed and run, residuals were much closer to having equal variance between needle retention and region. Part of the problem is that needle retention has a limited range, with a scale of 0.0-4.0, which should be taken into consideration.

Another PROC GLIMMIX analysis was run to test the difference between means for branch diameter and region. Again, data required the logit transformation to better meet the assumption of equal variance. Because diameter data had more opportunity to be continuous, the distribution was close to normal, however, a gamma distribution was found to fit the data slightly better. PROC GLIMMIX was also used to analyze differences in staining patterns by region. Similar to the other variables tested, this used a logit transformation with a gamma distribution.

The experimental design is constructed in such a way that nesting occurs within regions by sites. To account for nesting effects, these analyses only evaluate the differences between the various indices of disease severity and diameter and the regions, as oppose to examining variability between sites. However, if individual trees are perceived as the unit of analysis, then variability between sites could be shown by performing the same analysis of variance methods employed above. One anomaly within the data is the site PCB, which tended to have fewer connections than the sites within the same region (PCA and PCC).

Stepwise logistic regression was used in SAS to determine if any significant differences existed between staining patterns and the indices of disease severity and diameter. However, because these variables are confounded, careful interpretation must be used to evaluate results. Associations between variables and staining patterns might exist, but not at a significance level of  $p < 0.05$ .

## Results

Disease severity and stem diameters between regions:

The McDonald-Dunn sites had higher average needle retention than the Plum Creek sites ( $p=0.0002$  and Table 2), supporting the disease impact classification used for the sites. On average, trees within sites in the Coast Range tended to retain 3.7 years of needle cohorts while trees within the Coastal region tended to only retain 2.7 years. Trees in the Coast Range, on average, were holding onto needles a full year longer than trees in the Coastal Region. This is consistent with my hypothesis, and supports needle retention as an appropriate index of disease severity.

The data collected by the companion study suggested that the McDonald-Dunn sites also had significantly fewer infected needles and a smaller proportion of stomates occluded by pseudothecia (Appendix).

Branch diameter is not significantly different between the Coast Range and the Coastal region ( $p=0.1962$ ). This does not support the hypothesis that diameter would be positively correlated to healthy trees within sites containing higher needle retention. It was predicted that larger diameters might describe trees within sites exhibiting low SNC. However, this study found that diameter is not significantly different between regions and is not an appropriate index of disease severity for younger trees.

#### Patterns of stain in growth rings:

Data showed that there was not a significant difference in staining patterns between the Coast Range and the Coastal region ( $p=0.2636$ ). This does not support the hypothesis that trees within sites in the Coast Range would have more needle connections to subsequent years than trees within the Coastal region. However, trees within the Coastal region did have a tendency to make multiple connections, with the exception of site PCB. On average, 65% of all trees within the Coastal region formed new connections to wood produced in years subsequent to original needle attachment in the 2011 growth ring. In comparison, only 33.3% of all Coast Range trees made new connections (Table 2).

#### Correlations of disease severity indices and diameter with stain patterns:

Needle retention and diameter were not found to be significant predictors of staining patterns. A stepwise logistic regression analysis found that the variables of disease severity indices were not significantly correlated to staining patterns, in part due to variables being confounded (explaining the same variability between samples that stained only the 2011 growth ring and samples that made new connections). As long as “sites” are the unit of measurement for analysis, variables such as needle retention (which was found to be significantly different between regions) are nested within these sites and confound the results. Based on observation, there does seem to be a tendency for samples with lower needle retention to have more connections (Fig. 6), but this tendency was not found to be significant (at  $p<0.05$ ).



## Discussion

These results did not support the hypothesis that severely diseased SNC trees would have fewer needle connections than trees with little SNC impact. There was some variability among sites, but overall patterns of needle attachment between regions were not as expected. Needle retention was found to be significantly different between the Coast Range and the Coastal region, with trees in Coast Range sites tending to retain needles a year longer than trees in Coastal sites. There was no significant difference in branch diameter between the two regions. Needle retention and branch diameter were not seen to be significant predictors of needle attachment patterns in three year old needles (produced in 2011).

Heavily infected stands of SNC in the Pacific Northwest can be severely impacted, to the point where fewer than two years of needle cohorts are retained for any infected tree. This study used moderately severe stands (classified as high SNC here) for the purposes of testing needle attachment in three year old needles produced in 2011. To do this, samples from Coastal sites (where high SNC infection is known to occur) (Maguire et al. 2011) were required to have retained at least some of their 2011 needles so as to be tested in lab. If very heavily impacted sites had been used, needle retention would have been too low to sample and test three year old needles.

The variability in needle attachment patterns found across samples of Douglas-fir with varying degrees of disease severity is a significant finding that shows needle attachment isn't a fixed pattern even within one species and also across levels of disease severity. Variation in attachment occurs within a single stand and between different sites.

These inconsistent patterns of attachment are important to consider when evaluating the ascent of water for photosynthesis within an individual tree. Water availability could be different for different cohorts of needles, depending on the spatial variability of these connections within the sapwood, which likewise could have influences with variable water potentials. Other studies have found that water potentials are variable at different depths in the sapwood (Domec et al. 2005).

Needle attachment patterns are an important aspect to consider in understanding the possible effects of foliage disease, such as SNC, on trees. It is not a fixed arrangement, but rather something that changes and is possibly influenced by disease. Future research could benefit by taking the variability of needle attachment within and among species into consideration.

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## APPENDIX

This appendix contains original data from this project as well as the data collected in the companion study. Region is either the Coast Range which contained three sites (MA, MB, and MC) or Coastal (PCA, PCB, and PCC). Site indicates which of the six sites the sample is from. Tree ID is the identifier from which one branch sample was taken. NR is the needle retention measured in years. Diameter is of the diameter of the branch segment studied, measured under the bark on the dried samples. Stained Only 2011 is coded so that 1= yes (those samples that had stain in only the 2011 xylem) and 0= no (all other results). SNC incidence and percentage of blocked pseudothecia refer to the ongoing data collection from the companion study; these measurements are the total number of needles with pseudothecia present (50 needles/tree) and percent of stomates occluded by pseudothecia across 30 measurements (3 measurements/needle and 10 needles/tree), respectively.

Region	Site	Tree ID	NR (yrs)	Diameter (mm)	Stained Only 2011	SNC incidence	Blocked pseudothecia (%)
Coast Range	MA	1	4	2.405	0	7	7.904762
Coast Range	MA	2	4	2.46	1	46	17.733333
Coast Range	MA	3	4	3.935	0	47	15.66667
Coast Range	MA	4	4	2.09	1	48	15.66667
Coast Range	MA	5	4	4.84	0	36	5.7333333
Coast Range	MA	6	4	1.86	1	47	29.133333
Coast Range	MA	7	3.1	2.855	1	47	24.6
Coast Range	MA	8	3.7	4.24	0	44	9.4
Coast Range	MA	9	4	3.015	0	35	8.2
Coast Range	MA	10	3.8	1.79	1	20	16.06667
Coast Range	MA	11	4	3.5	0	14	9.3333333
Coast Range	MA	12	3.8	2.935	1	48	31
Coast Range	MA	13	4	3.9	1	36	22.4
Coast Range	MA	14	3.3	2.315	1	29	13.76667
Coast Range	MA	15	3.9	3.96	1	39	14.96667
Coast Range	MA	16	4	3.345	1		
Coast Range	MA	17	4	2.215	1		
Coast Range	MA	18	3.9	2.685	1		
Coast Range	MA	19	3.7	3.325	0		
Coast Range	MA	20	4	4.215	1		
Coast Range	MA	21	4	3.26	1		
Coast Range	MA	22	3.9	3.04	1		
Coast Range	MA	23	4	3.73	1		
Coast Range	MA	24	4	3.175	1		
Coast Range	MA	25	3.4	6.47	1		

Coast Range	MA	26	3.3	2.45	1		
Coast Range	MA	27	4	5.98	0		
Coast Range	MA	28	4	3.66	0		
Coast Range	MA	29	3.1	2.96	1		
Coast Range	MA	30	4	3.63	0		
Coast Range	MB	1	3.9	2.22	1		
Coast Range	MB	2	3.8	2.41	1	10	5.733333
Coast Range	MB	3	3.6	2.895	0	36	20
Coast Range	MB	4	3.2	3.33	0		
Coast Range	MB	5	3.5	3.4	0	12	28.46667
Coast Range	MB	6	3.1	3.595	0		
Coast Range	MB	7	3.9	1.82	0		
Coast Range	MB	8	4	3	0		
Coast Range	MB	9	3.9	1.56	1		
Coast Range	MB	10	3.7	3.515	1		
Coast Range	MB	11	3.7	1.535	1		
Coast Range	MB	12	3.5	2.85	1		
Coast Range	MB	13	3.2	2.935	1		
Coast Range	MB	14	3.8	1.885	1		
Coast Range	MB	15	3.7	4.455	1		
Coast Range	MC	1	3	3.055	0	46	22.73333
Coast Range	MC	2	3	3.36	0	50	17.63333
Coast Range	MC	3	3.7	3.755	1	17	
Coast Range	MC	4	3.7	1.795	1		
Coast Range	MC	5	3.6	3.695	1		
Coast Range	MC	6	3.9	2.255	1		
Coast Range	MC	7	3.9	2.04	1		
Coast Range	MC	8	3.8	3.64	1		
Coast Range	MC	9	3.9	3.265	0		
Coast Range	MC	10	4	2.37	1		
Coast Range	MC	11	4	2.7	1		
Coast Range	MC	12	3.9	2.09	1		
Coast Range	MC	13	3.5	3.25	1		
Coast Range	MC	14	3.9	2.56	0		
Coast Range	MC	15	4	3.915	1		
Coastal	PCA	1	2.8	2.14	0	50	53.8
Coastal	PCA	2	2.9	3.08	0	50	44.4
Coastal	PCA	3	2.5	3.11	1	50	55.13333
Coastal	PCA	4	3	3.52	0	50	29.3
Coastal	PCA	5	2.9	2.765	0	50	49.5
Coastal	PCA	6	2.5	6.03	0	50	45.8

Coastal	PCA	7	2.6	3.25	1	50	39.9
Coastal	PCA	8	2.4	3.735	0	50	40.16667
Coastal	PCA	9	3.1	3.25	0	50	42
Coastal	PCA	10	3.7	2.95	0	50	44.2
Coastal	PCA	11	2.6	4.045	1	50	39.3
Coastal	PCA	12	2.5	4.72	0	50	30.3
Coastal	PCA	13	3.4	2.36	0	50	42.33333
Coastal	PCA	14	2.3	2.485	0		
Coastal	PCA	15	2.3	5.185	0		
Coastal	PCA	16	2.7	3.275	0		
Coastal	PCA	17	3	4.085	0		
Coastal	PCA	18	2.8	3.775	0		
Coastal	PCA	19	2.4	2.945	0		
Coastal	PCA	20	2.8	2.505	0		
Coastal	PCA	21	2.9	1.2	0		
Coastal	PCA	22	2.1	3.965	0		
Coastal	PCA	23	2.9	3.82	0		
Coastal	PCA	24	2.7	3.435	0		
Coastal	PCA	25	2.9	2.765	0		
Coastal	PCA	26	2.8	2.89	0		
Coastal	PCA	27	2.5	3.325	0		
Coastal	PCA	28	2.1	4.64	0		
Coastal	PCA	29	2.8	2.585	1		
Coastal	PCA	30	3	2.835	1		
Coastal	PCB	1	3.8	2.015	1	50	37.66667
Coastal	PCB	2	3	3.115	1	50	30.23333
Coastal	PCB	3	3.1	3.87	1	49	27.73333
Coastal	PCB	4	2.5	4.035	0	50	23.33333
Coastal	PCB	5	2.6	2.745	1	50	37.3
Coastal	PCB	6	2.7	2.375	1	50	40.53333
Coastal	PCB	7	3.1	3.13	1	50	13.16667
Coastal	PCB	8	2.5	3.05	0	50	16.8
Coastal	PCB	9	2.9	2.515	1	50	29.6
Coastal	PCB	10	3.1	2.83	1	49	29.7
Coastal	PCB	11	2.6	3.69	1		
Coastal	PCB	12	2.5	3.805	1		
Coastal	PCB	13	0.5	5.695	0		
Coastal	PCB	14	2.8	4.155	1		
Coastal	PCB	15	2.5	2.68	1		
Coastal	PCC	1	2.9	4.335	0	50	49.8
Coastal	PCC	2	2.8	2.88	0	50	41.96667



Coastal	PCC	3	2.3	2.95	0	50	16.23333
Coastal	PCC	4	2.8	5.33	0		
Coastal	PCC	5	3.5	2.965	0		
Coastal	PCC	6	2.2	3.065	0		
Coastal	PCC	7	3	2.37	0		
Coastal	PCC	8	2.3	3.6	0		
Coastal	PCC	9	2.9	2.7	1		
Coastal	PCC	10	2.5	4.015	1		
Coastal	PCC	11	2.4	2.92	1		
Coastal	PCC	12	2.2	3.125	0		
Coastal	PCC	13	2.8	4.5	1		
Coastal	PCC	14	3.1	4.02	0		
Coastal	PCC	15	2.3	3.19	0		

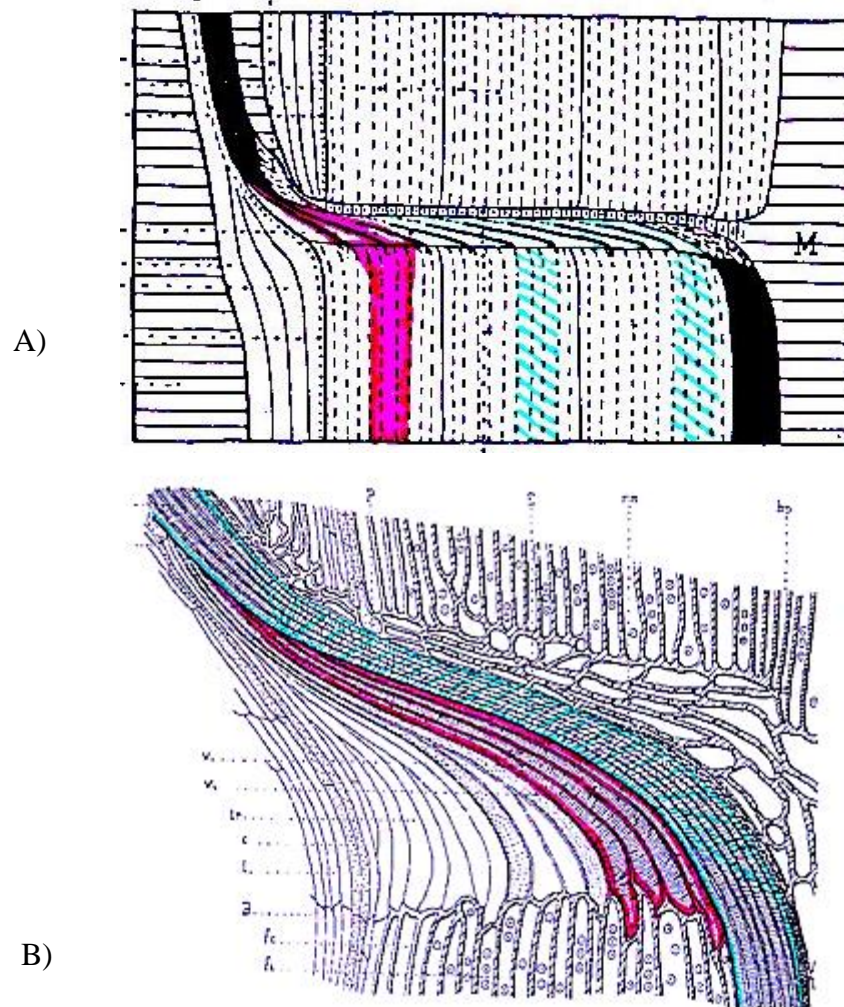


Fig. 1. A) Needle traces (above m) and stem xylem from which needle withdrew water (below m) in a stem with four growth rings. Longitudinal view through stem, with bark on left and pith on right. B) Anatomy of needle traces. Magenta indicates current year functional needle trace, turquoise indicates broken, non-functioning needle trace from previous years (seen by broken and stretched tracheids). (Modified from Tison 1903)

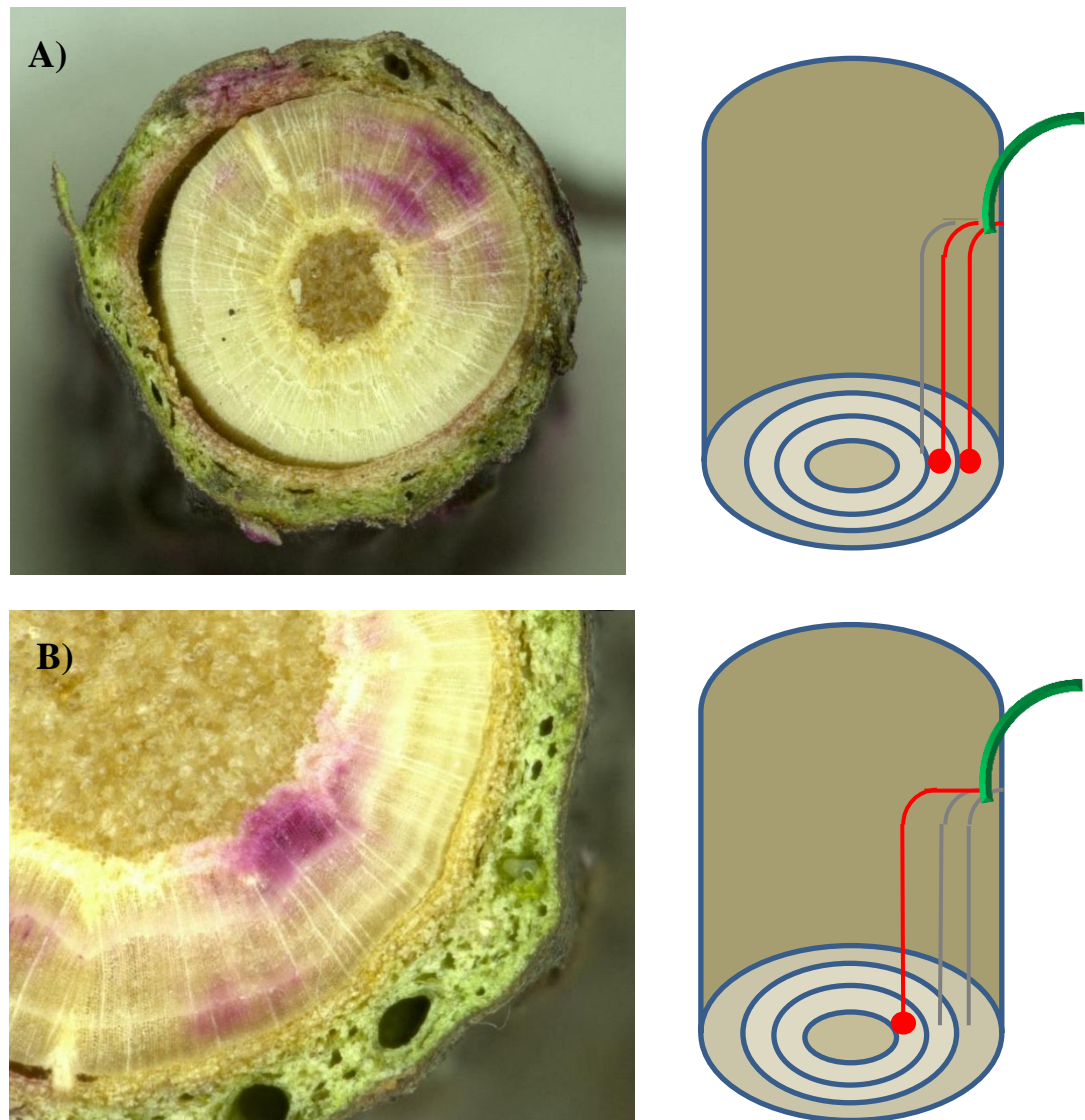


Fig. 2. A) Staining patterns of three-year old stems (developed in 2011, 2012, and 2013), with both a photograph and a schematic indicating stain pathways. Needle developed in 2011 drew stain through A) the 2012 and 2013 growth rings and B) the 2011 growth ring.

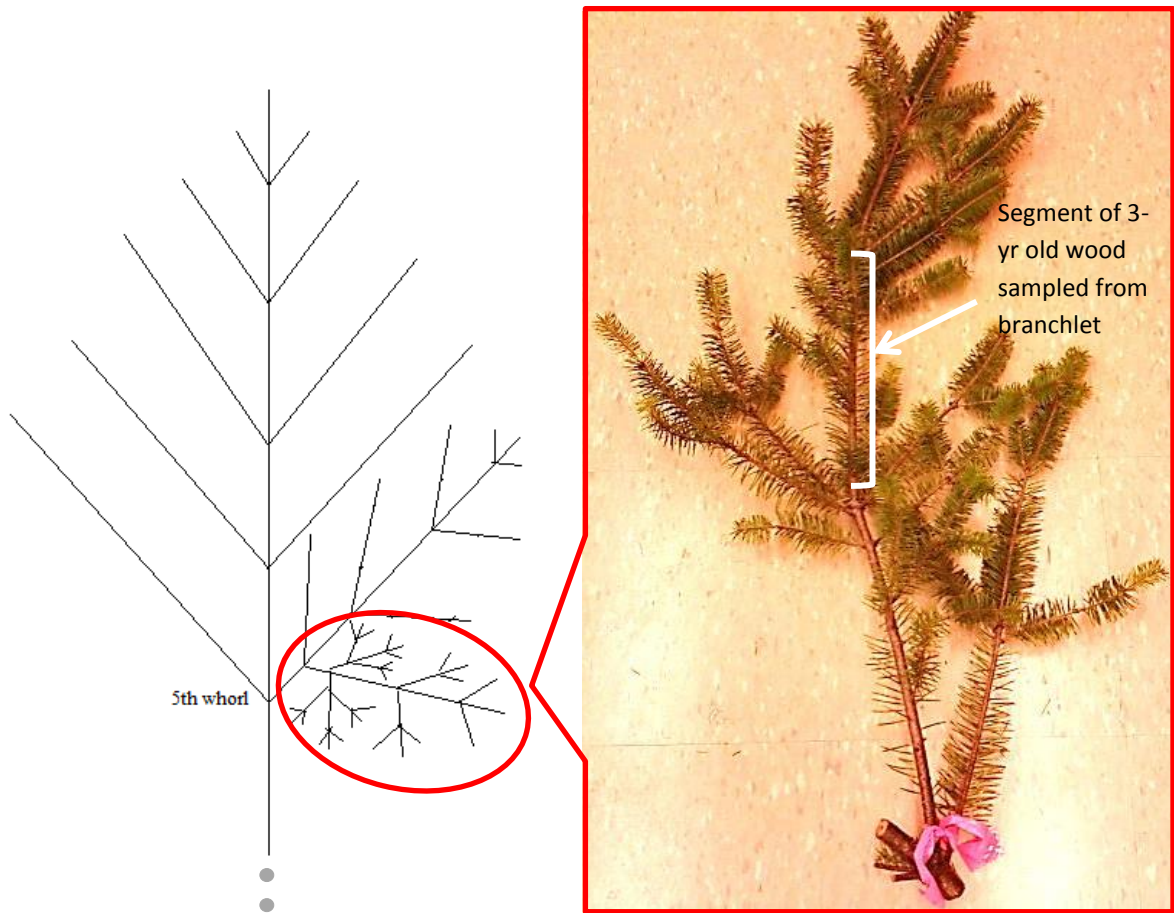


Fig. 3. Sample location in tree. A) Schematic design of the top five whorls (years) of the Douglas-fir tree. B) Photograph of branch location from which sample was taken.

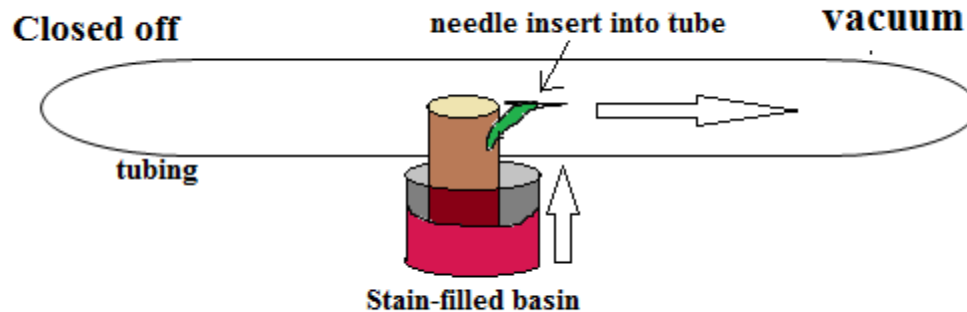


Fig. 4. Experimental set up. Method for pulling stain through stem segment into needle. The tip of target needle that developed in 2011 was removed. The remainder of the needle tip was then enclosed into a tube through which a vacuum was applied. The sample's vase was set in a basin of 1% aqueous acid fuchsin, and allowed to run for five minutes.

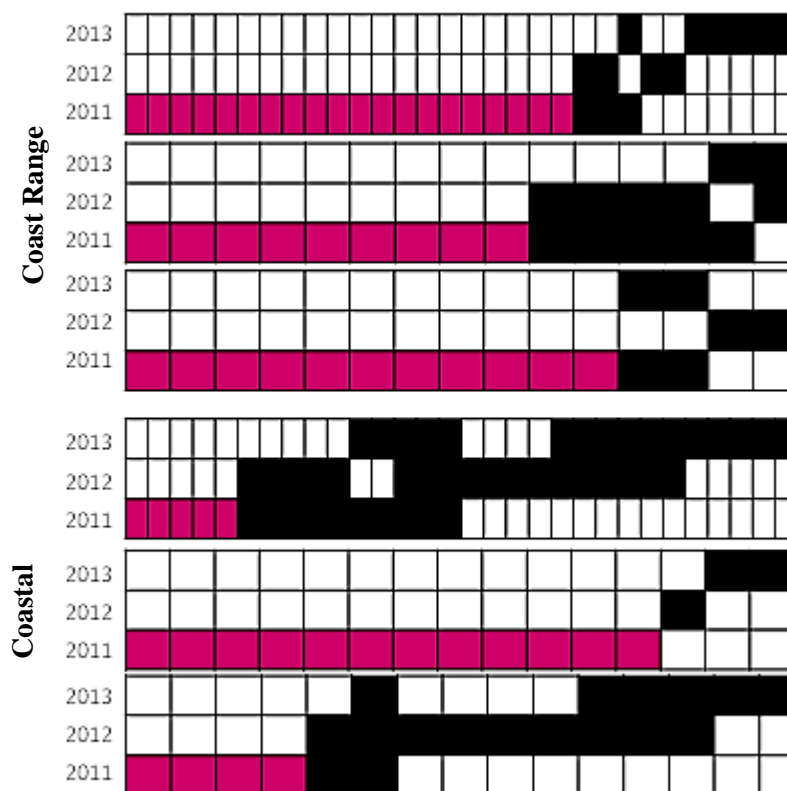


Fig. 5. Staining results. Each sample is shown as a vertical stack of three boxes, representing the stem xylem produced in 2011, 2012, and 2013. Each array represents one site (MA, MB, MC, PCA, PCB, and PCC from top to bottom). The box is colored if stain was observed in that growth ring. For samples in which only the 2011 growth ring stained, the boxes are colored magenta.

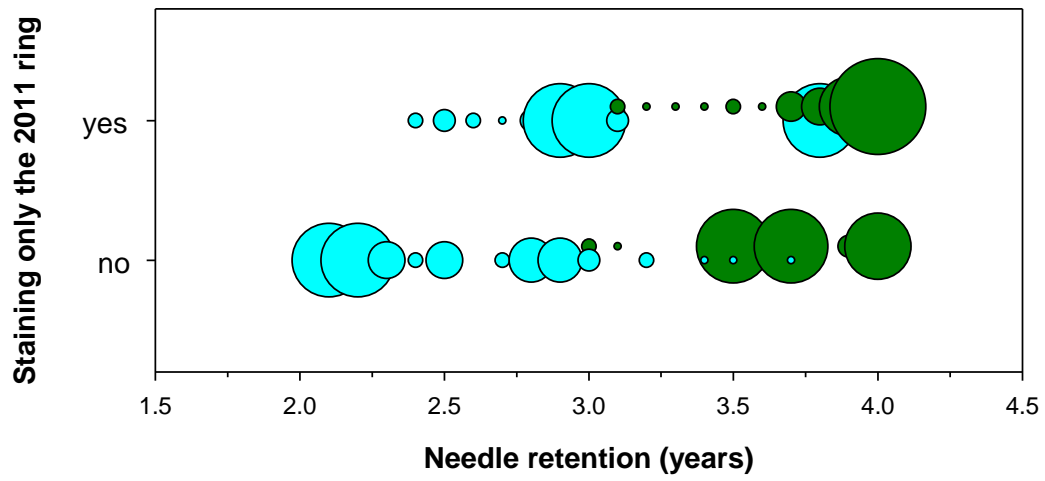


Fig. 6. Staining results across needle retention. Cyan represents samples within Coastal sites and green represents samples within Coast Range sites. Relative size of the bubbles indicates number of samples.

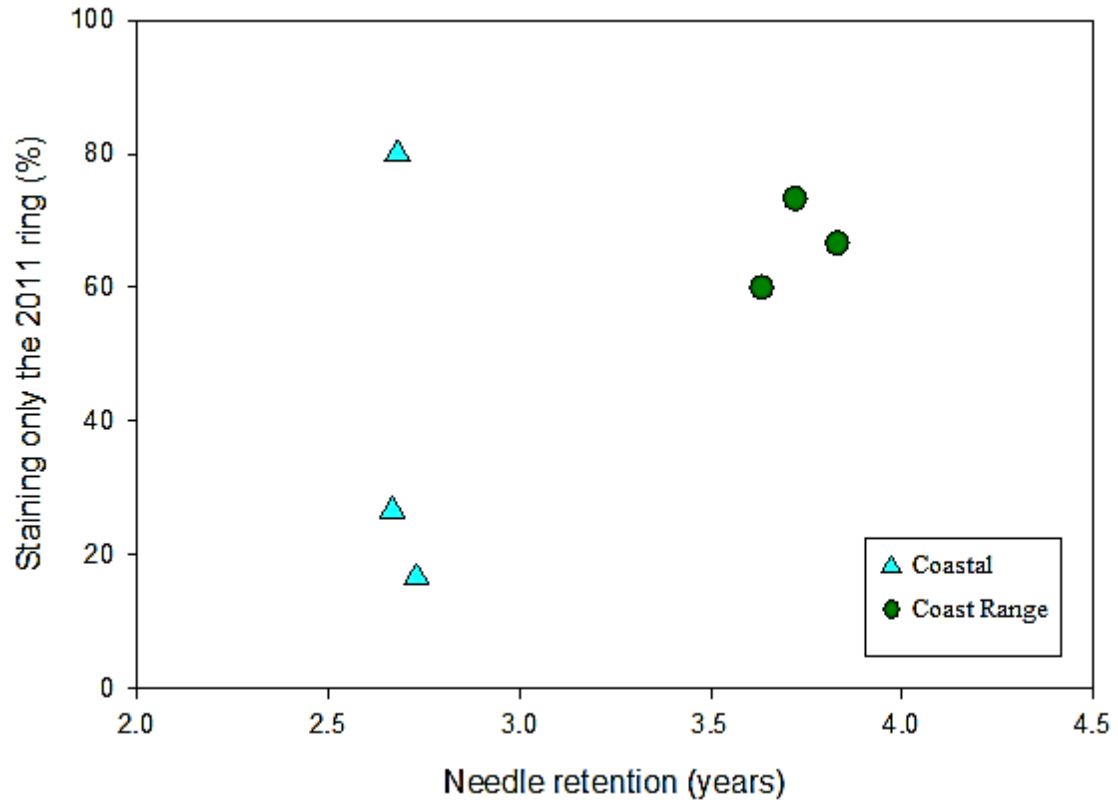


Fig. 7. Staining patterns of sites within regions across needle retention. The lower the proportion for staining in only the 2011 growth ring, the greater the tendency for a needle to have formed new connections.



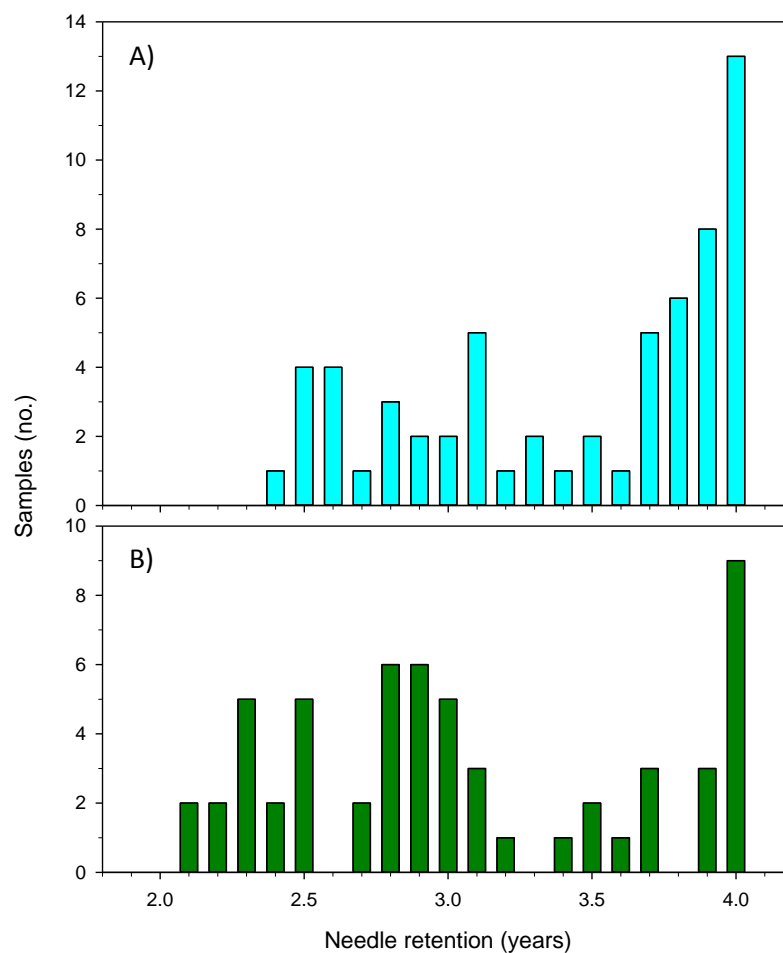


Fig. 8. Staining pattern across needle retention for all pooled samples. Plots show A) number of samples that stained only 2011 growth ring and B) number of samples that exhibited all other staining patterns.

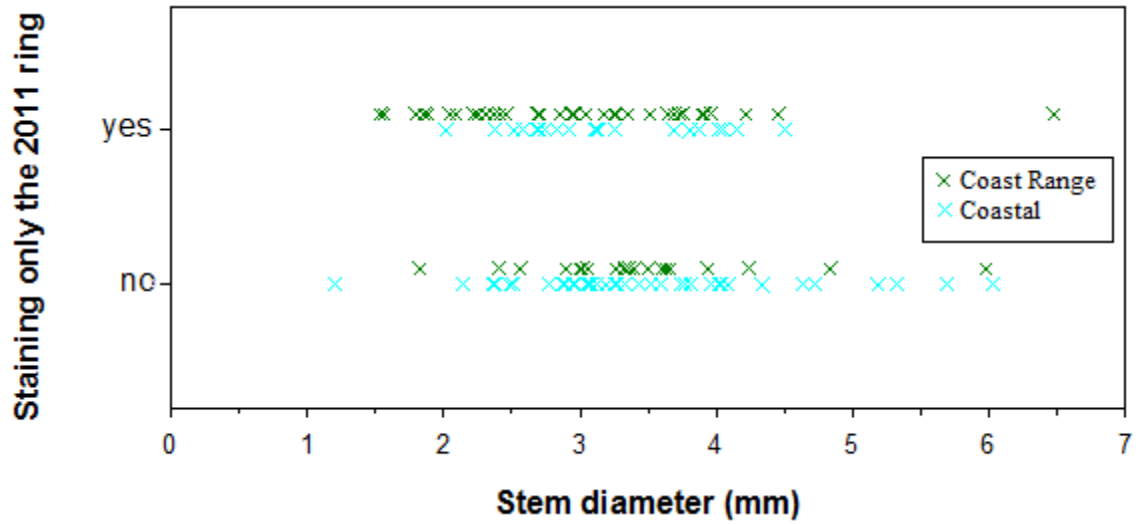


Fig. 9. Individual tree data of staining patterns by diameter within regions. Plot shows variability in diameter within regions, but no significant difference between the Coast Range and Coastal regions.

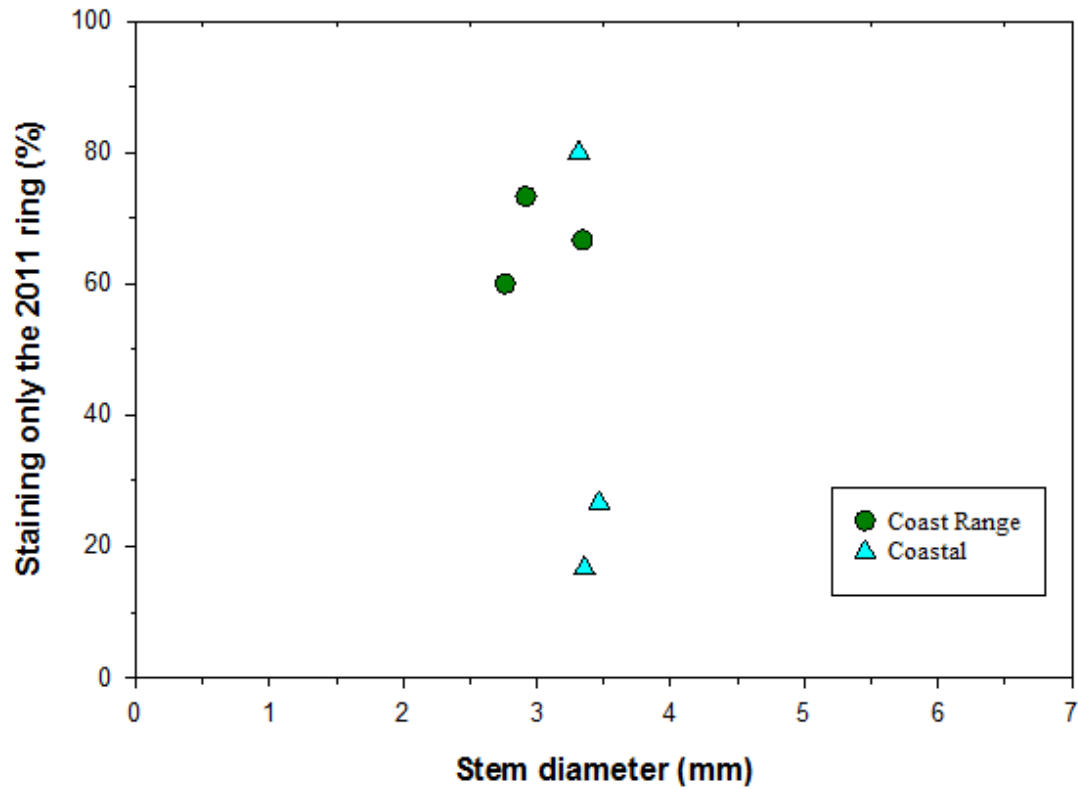


Fig. 10. Staining pattern and stem diameter averaged within sites and across regions. There seemed to be no significant relationship between stem diameter and staining pattern. Within regions, however, there was a tendency for Coast Range sites to have a higher proportion of staining in only the 2011 xylem relative to Coastal sites.

Table 1. Description of study sites. Stand age is years from planting.

Region	Site Designation	Stand Name	Stand Age (yrs)	Ownership	Location
Coast Range	MA	Good Neighbor	9	McDonald-Dunn Forest, College of Forestry, OSU	Corvallis, OR 44.608°N, -123.336°W, 172m
Coast Range	MB	Wapiti	9	McDonald-Dunn Forest, College of Forestry, OSU	Corvallis, OR 44.698°N, -123.275°W, 120m
Coast Range	MC	Yellow Jacket	9	McDonald-Dunn Forest, College of Forestry, OSU	Corvallis, OR 44.713°N, -123.317°W, 150m
Coast	PCA	unknown	9	Plum Creek Timber	Toledo, OR 44.678°N, -124.000°W, 217m
Coast	PCB	unknown	9	Plum Creek Timber	Toledo, OR 44.695°N, -123.978°W, 160m
Coast	PCC	unknown	9	Plum Creek Timber	Toledo, OR 44.711°N, -124.005°W, 198m

Table 2. Measures of SNC disease severity, stem diameter, and staining in only 2011 growth ring in the six sites sampled. The McDonald-Dunn (Coast Range) sites are on the eastern flank of the Coast Range, and owned by the College of Forestry, Oregon State University. The Plum Creek (Coastal) sites are on the western flank of the Coast Range within 10 km of the ocean, and owned by Plum Creek Timber. (Mean  $\pm$  s.e. (n)) Subscripts signify significant differences between sites. A site with the designation <sub>a</sub> is significantly different than a site with <sub>b</sub>.

Region	Sites	Assumed SNC impact	Needle retention (years)	Stem diameter (mm)	Staining only in 2011(%)
Coast Range	MA	low	3.8 $\pm$ 0.1 (30)	3.3 $\pm$ 0.2 (30)	66.7
Coast Range	MB	low	3.6 $\pm$ 0.1 (15)	2.8 $\pm$ 0.2 (15)	60.0
Coast Range	MC	low	3.7 $\pm$ 0.1 (15)	2.9 $\pm$ 0.2 (15)	73.3
Coastal	PCA	high	2.7 $\pm$ 0.1 (30)	3.4 $\pm$ 0.2 (30)	16.6
Coastal	PCB	high	2.7 $\pm$ 0.2 (15)	3.3 $\pm$ 0.2 (15)	80.0
Coastal	PCC	high	2.7 $\pm$ 0.1 (15)	3.1 $\pm$ 0.2 (15)	26.7
Coast Range		low	3.7 $\pm$ (3) <sub>a</sub>	3.1 $\pm$ 0.1(3) <sub>a</sub>	66.7 <sub>a</sub>
Coastal		high	2.7 $\pm$ (3) <sub>b</sub>	3.4 $\pm$ 0.1 (3) <sub>a</sub>	35.0 <sub>a</sub>

