AN ABSTRACT OF THE DISSERTATION OF

Yung-Hsiang Lan for the degree of Doctor of Philosophy in Forest Ecosystems and Society presented on May 31, 2018.

Title: Canopy Ecology of Swiss Needle Cast in Young and Mature/Old-growth Douglas-fir (*Pseudotsuga menziesii*)

Abstract approved:

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Swiss needle cast (SNC) is a foliage disease of Douglas-fir caused by *Nothophaeocryptopus gaeumannii*, an Ascomycete fungus (Mycosphaerellaceae) that causes growth reductions in Douglas-fir plantations across the Pacific Northwest. Epidemiology of the fungus is generally well known in plantation trees, but the relationship between disease expression and foliage nutrition and some climate variables is unclear. While the dynamics of SNC in older trees is also poorly understood.

In Chapter 2, data from the Swiss Needle Cast Cooperative (SNCC) research and monitoring plot network across western Oregon and SW Washington State was utilized to assess the associations between disease severity, needle retention, carbon, and 9 foliage nutrients (N, Na, K, P, Ca, Mg, Mn, Al, and S). Foliage samples were collected from upper, mid and lower crowns of five Douglas-firs from each plot. SNC disease severity was determined from 2-year old needles by multiplying the ratio of occluded stomates by the percentage of needles with fungal reproductive structures (pseudothecia) for the 50 needles. SNC disease severity and needle retention were more highly associated in the mid crown than in upper and lower crown. Mid-crown SNC disease severity and nutrient relationships were determined using linear mixed models. SNC disease severity showed statistically significant positive trends with C ($p<0.001$), N ($p<0.001$), Na ($p<0.001$), K ($p=0.004$), S ($p<0.001$), no relationship with Ca, Mg, or Al, and slightly negative trends that were not significant for P and Mn. Although some nutrients were associated with increasing SNC disease severity, more research is required to determine the cause-effect.

In Chapter 3, climatic factors, which strongly influence epidemiology, intensification and impacts of disease on tree growth, are considered. Our study was conducted in 106 systematically placed research plots established by the Swiss Needle Cast Cooperative in 2013-2015. Climate variables tested were monthly and annual precipitation, minimum temperature, maximum temperature, mean temperature, mean dew point temperature, and maximum VPD. We also examined the influence of latitude and longitude on climate variables and severity of SNC. Minimum temperature and dew point temperature were the most significant factors related to SNC disease severity ($p<0.001$). Oct-Apr mean temperature, Oct-Apr maximum temperature, and Nov-Apr maximum VPD were also associated with SNC disease severity ($p<0.001$). Monthly precipitation was not associated with mean SNC disease severity during the summer months. Dew point temperature for all months was positively associated with SNC disease severity ($p<0.001$). We suggest that dew point temperature may be more important in epidemiology of *N. gaeumannii* than previously thought. Latitude had a strong relationship with SNC disease severity and climate variables, while longitude did not. Analyses of climate relationships within subregions in the study area indicated that relationships between SNC disease severity and climate variables were strongest in the Tillamook region of northwest Oregon.

In Chapter 4, while there is considerable evidence of SNC disease in coastal Douglas-fir plantations, the severity of SNC in mature and old-growth forests is poorly understood. We compared the SNC severity, incidence, needle retention, and foliar nitrogen in tree crowns of mature and old-growth forests and nearby young forests at three locations in the Oregon Coast Range and four locations in the western Cascade Mountains of Oregon. Disease severity, as assessed on 2-year old needles, was greater in younger forests than older forests at all sites. Retention of 1-4 year-old needle cohorts did not differ between young and old trees, but older trees had much larger complements of >4 year-old needles. Incidence of disease was highest for 2-year-old needles in young trees and 3-5 year-old needles in older trees. Total foliar nitrogen concentration did not differ in needles of young and old trees, but at some locations total N differed between canopy positions. Leaf wetness differences were not consistent between young and old tree crowns and did not explain disease severity differences. However, at a study site in the core epidemic area, the younger stand had longer periods of wetness in the upper crowns than a nearby old stand. Leaf wetness and foliar N were hypothesized to play a role in SNC disease severity, but apparently these are not controlling factors. In younger stands, the fungus appeared to block stomates earlier than in older stands and stomatal occlusion was always greater on younger than older trees for 2 year old needles. We speculate that multiple factors may have caused the observed differences, including differences in thermal properties

of older and younger stands, needle anatomy, chemical differences, or genetics of old tree and young trees. It is also possible that older trees are less impacted by SNC because they have experienced exposure to the disease over a longer period of time, and this influences host-fungus interactions. Also, four of the sites that we examined were outside the current epidemic area and were not considered diseased, so this may have influenced needle retention. The relationships observed in our study need testing with larger samples to determine if our results are generally applicable to Douglas-fir in western Oregon.

From these studies, we tested nutrient and climate variables, which narrowed down the potential factors associated with SNC for further focused modeling. Also, we provided quantitative and qualitative description of SNC patterns comparing mature and young tress, and suggested more research about SNC in mature and old-growth Douglas-fir forests in needed because of the potential of SNC to influence stands of all ages.

Canopy Ecology of Swiss Needle Cast in Young and Mature/Old-growth Douglas-fir (*Pseudotsuga menziesii*)

by
Yung-Hsiang Lan

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Dean of the Graduate School

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

Yung-Hsiang Lan, Author

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CONTRIBUTION OF AUTHORS

Dave Shaw and Yung-Hsiang Lan designed studies in this dissertation. Yung-Hsiang Lan contributed most of data analysis and writing in the five chapters of this dissertation with the critical revision by Dave Shaw.

Gabi Ritóková installed and organized SNCC research and monitoring plot network, produced database used in Chapter 2 and Chapter 3. Gabi Ritóková and Jeff Hatten helped on interpreting data and revisions on Chapter 2 and Chapter 3. Jeff Hatten also contributed the chemical analysis in Chapter 2.

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CHAPTER 1-GENERAL INTRODUCTION: STOP BREATHING! SWISS NEEDLE CAST ON DOUGLAS-FIR

Spring is a beautiful season. Blooming is everywhere. For some, however, pollen may cause health issues. Pollens can trigger our immune systems causing nasal congestion, sneezing, wheezing, and even asthma or fevers. For allergy sufferers, it is horrible to have so many tiny pollen grains block your nostril and lungs.

Douglas-fir (*Pseudotsuga menziesii*), one of the most valued timber species in Pacific Northwest of the US, has the similar issues during spring. If you walk into the forest and pick up a Douglas-fir needle, turn it over and examine it with a hand lens, you might be able to see some signs of distress. You might see some black and white dots on the needle (Fig. 1). The white dots are air pores called stomates, an important leaf structure. Just as like a human nostril, plants have stomates which open to breathe, and allow CO_2 to enter the leaf for photosynthesis (the reaction fixing carbon from the air to plants). The stomates are lined up regularly like theater seats, but you can see there are some black dots between them – some seats seem occupied (Fig. 1.1).

The black dots are *Nothophaeocryptopus gaeumannii*, a native fungus in the Pacific Northwest. *N. gaeumannii* is endophytic, which means it grows within needles without causing problems until the stomates are plugged. The fungus only lives on Douglas-fir. Although it is a fungus, though unlike general mushrooms, *N. gaeumannii* does not have obvious reproductive structures. Instead, it has a tiny ball-like structure called pseudothecia, which are the black dots (Fig. 1.1), containing numerous spores inside which will be released after maturation. From late May through August, pseudothecia will mature and become visible with a hand lens on Douglas-fir needles. Then spores are released. Landing on the newly emerged needle surface, they germinate and the fungus grows into needles via the stomates. In the following year, the fungus grows within needles until it matures and produces the pseudothecia, which block air coming into the needle and water leaving the needle.

As seen in Figure 1.1, the peudothecia is approximately the same size and shape as stomates, which means it can perfectly "plug" the stomates physically. Stomates are the structure for plant to breathe, therefore, the stomates lose their function when peudothecia plug them. If 50% of the stomates of a needle are blocked by pseudothecia, the needle will be cast from trees because it cannot be used for gas exchange (Hansen et al. 2000). For most severe stands, needle loss can cause upwards of 52% annual growth compared to other healthy trees (Maguire 2002). Douglas-fir can be infected by the fungus can occur without causing disease, but when we see obvious needle loss, yellowish needle during spring, or growth decreases (Fig. 1.2), it is called Swiss needle cast, which is a widespread foliage disease along the west coast of Oregon and Washington. Although *N. gaeumannii* is a native fungus in North America, the disease was first reported in Switzerland in 1925 when Douglas-fir was introduced as plantation tree in Europe.

N. gaeumannii is the pathogen causing Swiss needle cast, however, it is only called a "disease" when the symptoms become visible. Just as bacteria and virus are everywhere in nature but not always causing disease, *N. gaeumannii* is present most Douglas-fir forests in coastal Oregon and Washington, but not always causing Swiss needle cast which results in large amounts of defoliation and growth decreases. Because of that, looking for the fungus presence is not a good way to represent the disease severity. In order to determine disease severity, scientists use 2-year-old needles as the material to evaluate the percentage of fungus presence and the intensity of *N. gaeumannii* occupation, then combine them as the severity index (Manter et al. 2005).

Swiss needle cast was not considered as a serious issue in Douglas-fir plantations historically. However, it became a bigger problem in the 1990s because we found much more infection in Pacific Northwest coastal area than before. From the aerial survey by US Forest Service and State Forestry Agencies via looking for visible symptoms, Swiss needle cast increased to 238,705 ha in 2015, while it only was 53,050 ha in 1996 (Ritóková et al. 2016).

As a fungus, although *N. gaeumannii* does not look like a typical mushroom, temperature and humidity are important for its reproduction (Rosso and Hansen 2003), especially summer precipitation and winter temperature because of the fungus life cycle (Wilhelmi et al. 2017). With wetter summer and warmer winter, Swiss needle cast is most severe along the Oregon coast.

Scientists are worried about how climate change might cause more Swiss needle cast in North America (Lee el al. 2017). Dendrochronology, which is a subject studying tree growth data along time, is used for understanding the Swiss needle cast history. Because trees grow bigger and taller annually, scientists relate tree growth to the growth year, and inform other events like climate, hydrology or insect outbreak, to study which factor contributes to the tree growth. By using dendrochronology technique to analyze the relationship between tree growth, weather, and Swiss needle cast, Lee et al. (2017) found that trees in low and mid elevation in west slope of the Cascade Mountain Range had severe Swiss needle cast impacts in 1984-1986 because of the warmer winters and wetter summers, while tree growth had been suppressed by Swiss needle cast later in 1990s in high elevation area. The findings suggested that warmer winter and wetter summer associated with global warming may increase Swiss needle cast in Pacific Northwest. My research found that dew point temperature is much more important to SNC severity than previously thought and perhaps dew occurrence is also important.

Just as protein is an important nutrition for human living, nitrogen is a limiting and crucial element for tree health. Nitrogen deficiency may result in poor growth and defoliation, and affects many chemical reactions within plant because nitrogen is involving many biosynthesis pathways by forming enzymes. Because nitrogen is an element and invisible to the human eye, stable isotopes are used for studying the nutrient relationship between fungus *N. gaeumannii* and host Douglas-fir. Stable isotopes (for example, ^{15}N and ^{14}N) are the same chemical element but with different weights, resulting to the same chemical properties but different physical characteristics. Because ^{14}N is common present in nature, by providing ^{15}N and detecting the ratio of $^{15}N/^{14}N$, scientists found a higher percentage of nitrogen in fungal pseudothecia was from the host needles, and then suggested that *N. gaeumannii* might acquire nitrogen and carbon from Douglas-fir needles (Kavanagh et al. 2003). Even though nitrogen is related to Swiss needle cast disease severity, however, nitrogen fertilization did not increase or decrease the Swiss needle cast (Mulvey et al 2013). However, it was found that there is a positive correlation between the amount of nitrogen in the needle and increasing disease severity.

Human babies get sick easily. We build our immune systems over decades. Based on my observations, mature Douglas-fir trees have fewer problems with *N. gaeumannii* than young Douglas-fir, meaning that Swiss needle cast - though present everywhere – is less severe on mature trees than young trees. It is unclear why the young trees have been more affected by *N. gaeumannii*. This difference may be associated with forest structure, needle morphology, or different defense strategies.

When forests get older, the most obvious difference compared to young forests is vertical complexity of tree structure. Old-growth forests have higher biodiversity in plants, invertebrates and vertebrates, and microorganisms (Franklin et al. 1991). The complexity of forest structure may result in more diverse micro-environments, affecting small scale "climate" on needles and causing differences in temperature and humidity between mature and young Douglas-fir; therefore their susceptibility to *N. gaeumannii* may be different.

Scientists have found that the morphology of needles is age-related. Compared to young trees, needles from older trees were longer and wider (Day et al. 2001), and with a thicker cuticle (England and Attiwill 2006). Also, in old trees and in young trees, different chemicals are used for defense from pathogen (Erwin et al. 2001). The physical and chemical difference between old trees and young trees might be associated with how easily they can be accessed by a pathogen.

Although Swiss needle cast is considered a problem in the Pacific Northwest coastal plantations, it usually does not cause tree death. It may take a couple of years to recover the annual growth after a severe infection because the trees lost a large number of their needles for growth production. By getting rid of the infected needles, Douglas-fir will have a chance to breathe again with the better, healthier new needles.

The next time you travel along the Oregon and Washington coasts, you may be able to see the yellow crown and sparse needles of Douglas-fir on the roadside. What you are seeing is a common occurrence that Douglas-fir is suffering from fungal infection. If the young trees can recover from the breathing problems, they will survive as adults.

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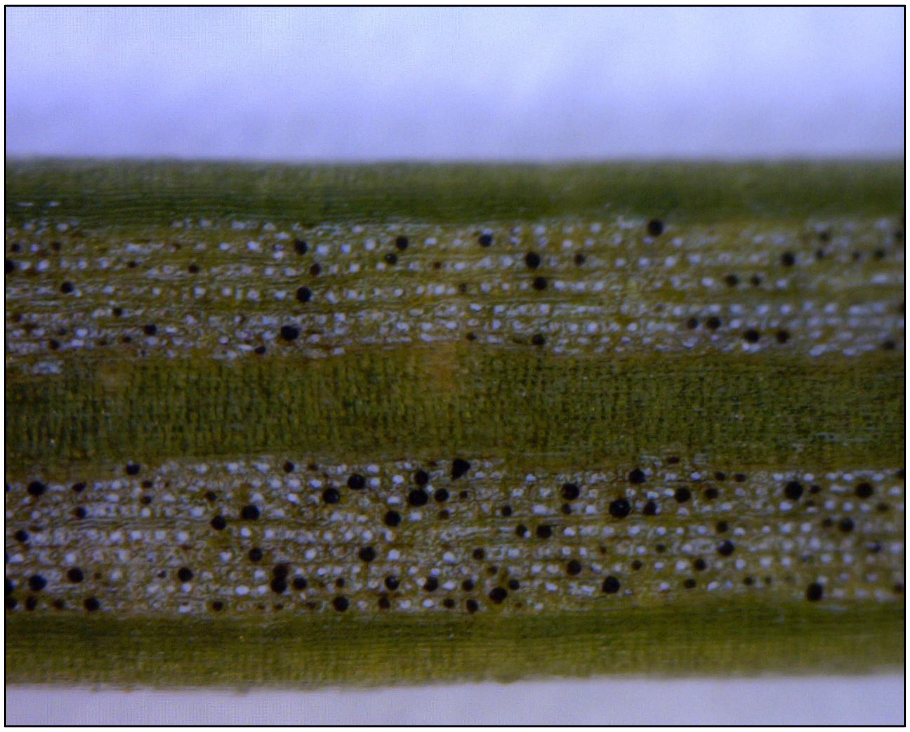

Figure 1.1. *Nothophaeocryptopus gaeumannii* blocking Douglas-fir air pores (stomates). (Photo credit: Swiss Needle Cast Cooperative http://sncc.forestry.oregonstate.edu/)

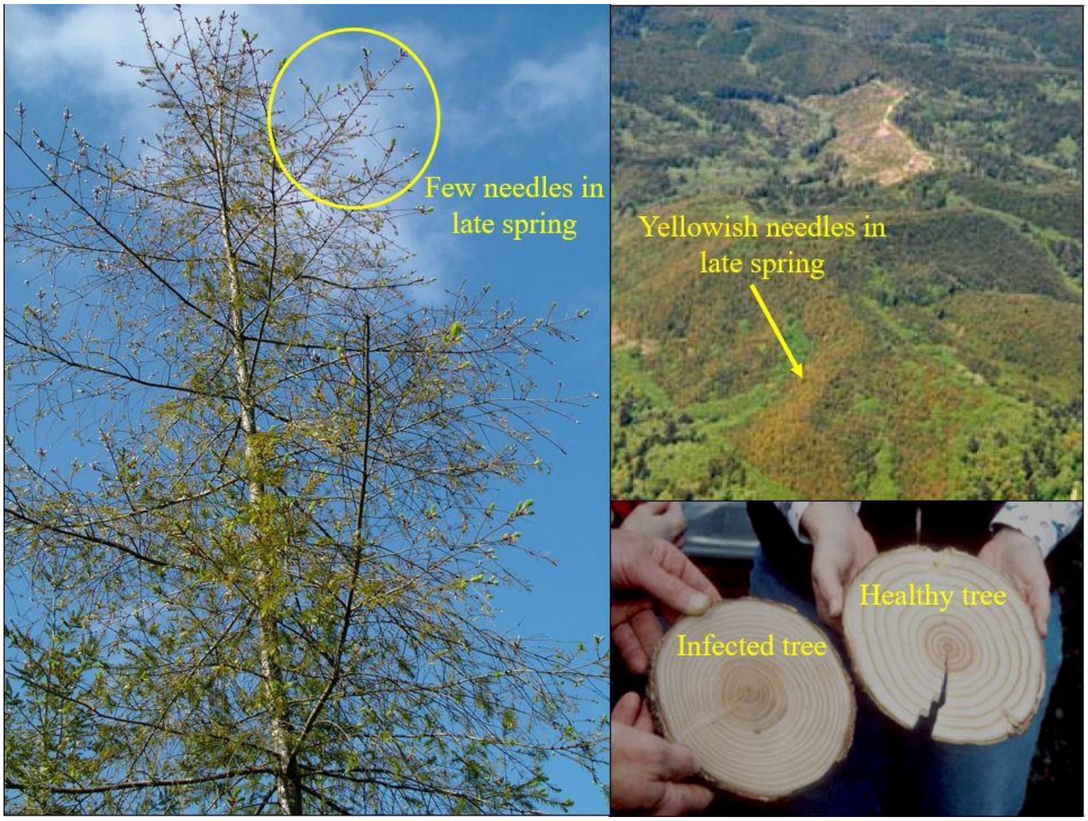

Figure 1.2. The typical symptoms of Swiss needle cast on Douglas-fir plantation. (Photo credit: Swiss Needle Cast Cooperative http://sncc.forestry.oregonstate.edu/)

CHAPTER 2 - ASSOCIATIONS BETWEEN SWISS NEEDLE CAST SEVERITY AND FOLIAR NUTRIENTS IN YOUNG-GROWTH DOUGLARS-FIR IN COASTAL WESTERN OREGON AND SOUTHERNWEST WASHINGTON, USA

Abstract

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii*) caused by *Nothophaeocryptopus gaeumannii*, an ascomycete fungus (Mycosphaerellaceae) that causes growth reductions in Douglas-fir plantations in the Pacific Northwest. Epidemiology of the fungus is generally well known, but relationships between disease expression and foliage nutrition is unclear. In this study, we used data from the Swiss Needle Cast Cooperative (SNCC) research and monitoring plot network in western Oregon and SW Washington to assess associations between SNC disease severity and multiple factors, including needle retention, carbon, and 9 foliage nutrients (N, Na, K, P, Ca, Mg, Mn, Al, S). Foliage samples were collected from upper, mid and lower crowns of five Douglas-firs from each plot. SNC disease severity was determined from 2-year old needles by multiplying % of stomates plugged by fungal reproductive structures (pseudothecia) and the incidence of 50 needles with any presence of pseudothecia. SNC disease severity and needle retention were more highly associated in the mid crown than in upper and lower crown. Mid-crown SNC disease severity and nutrient relationships were determined using linear mixed models. SNC disease severity showed statistically significant positive trends with C ($p<0.001$), N ($p<0.001$), Na ($p<0.001$), K ($p=0.004$), S ($p<0.001$), no relationship with Ca, Mg, or Al, and slightly negative trends that were not significant for P and Mn. Although some nutrients were associated with increasing SNC disease severity, more research is required to determine the cause-effect.

Introduction

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) caused by a native fungus, *Nothophaeocryptopus gaeumannii* (T. Rohde) Videira et al. (Ascomycete: Mycosphaerellaceae) (Videira et al. 2017). This disease causes growth losses in Douglas-fir plantations across coastal Oregon and Washington (Shaw et al. 2011). SNC was unimportant in forest plantations until the 1990s when it emerged as a significant concern (Hansen et al. 2000). The first aerial survey conducted in 1996 detected 53,050 ha of forest with visible disease symptoms in coastal Oregon. A resurvey of the same area in 2015 indicated that the area with visible symptoms of SNC had increased to 238,705 ha (Ritóková et al. 2016) and SNC is now seen as a significant threat to Douglas-fir growth. Although mortality from SNC is rare, growth loss of wood volume was estimated at ~23% within the NW Coast Range portion of the epidemic area. Some plantations experienced up to 52% growth losses (Maguire et al. 2002). Management of SNC requires a nuanced approach, because impacts vary with geographical setting, climate, and other plantation attributes.

Nothophaeocryptopus gaeumannii is a widespread, endophytic-fungus that causes visible disease when the stomates are plugged by fungal reproductive structures (pseudothecia). Needles with 50% or more plugged stomates will likely die and fall (“cast”) from the stem (Hansen et al. 2000). Overall needle retention of less than 3 years may cause reduced growth, while the tree may also show chlorosis (Maguire et al. 2011). Disease severity is usually determined by assessment of 2-year-old needles because this age class is best correlated with a disease effect (Manter et al. 2005). If pseudothecia occur on needles older than 4 years, there is no significant disease expression or growth impacts.

The potential for *N. gaeumannii* to cause disease is strongly influenced by temperature and moisture (Rosso and Hansen 2003, Manter et al. 2005). Attributes of seasonal temperature, especially winter temperature, may allow rapid development of the fungus within the needle. In addition, late spring and summer temperatures can influence spore germination and initial colonization of needles. Precipitation or atmospheric conditions that lead to leaf wetness during the spore dispersal period (May – August) are also necessary for successful colonization of needles by the fungus. In particular, precipitation or high humidity in June and July are associated with infection severity of *N. gaeumannii* (Rosso and Hansen 2003).

The role of N and Ca in the soil are hypothesized to be important in SNC disease (Perakis et al. 2006, El-Hajj et al. 2004, Mulvey et al. 2013). Perakis et al. (2006) showed a correlation between increased soil N and decreased soil Ca following the pattern of increasing SNC disease east to west across the Oregon Coast Range. El-Hajj et al. (2004) suggested that *N. gaeumannii* might acquire nitrogen and carbon from apoplastic spaces within Douglas-fir needles. In the El-Hajj et al. (2004) study, 10–year-old trees were fertilized and the authors found a positive association between the concentration of nitrogen in conifer needles and in the pseudothecia of the fungus, but there was only a weak relationship between carbohydrates in pseudothecia and infected needles.

El-Hajj et al. (2004) hypothesized that fertilization might be associated with increasing nitrogen availability in Douglas-fir forests. In their study, *N. gaeumannii* disease severity was positively associated with host nutrient levels, especially increased nitrogen. In contrast, Mulvey et al. (2013) found no increase in disease severity associated with standard amounts of fertilization with N, Ca, Mg, P, K or custom blends of fertilizers in the Oregon Coast Range, even though there were increases detected in foliage nutrient levels. Therefore, relationships between foliage nutrition and SNC disease remain equivocal.

In this study, we examined relationships between SNC severity and a variety of foliage nutrients in a network of research plots established by the Swiss Needle Cast Cooperative (SNCC) (http://sncc.forestry.oregonstate.edu/) in western Oregon and southwest Washington. The plot network included 106 plots in 10-30-year-old Douglas-fir plantations, systematically located within 56 km (35 miles) of the Pacific Ocean from the California border to southwest Washington (Fig. 2.1). The primary hypothesis that we addressed was, "Are foliage nutrient levels in Douglas-fir related to disease severity caused by *N. gaeumannii*?" Based on the results of a previous study (Perakis et al. 2006), we hypothesized that N and Ca would be strongly related to SNC severity. We also examined relationships between SNC severity and concentrations of other elements, including carbon, Na, K, P, Mg, Mn, Al, and S.

Methods

The 106 SNCC study plots were installed during 2013-2015, including 98 plots in western Oregon and 8 plots in southwestern Washington (Fig. 2.1). Each plot consisted of a 1/5-acre (0.08 ha) of uniform-age trees 10-25 years old. The targeted basal area composition was 80% Douglas-fir and 300-400 trees per acre. All trees were tagged and measured for diameter at 137 cm, and a subset of 40 trees were measured for height. All plots were established in plantations that had not been pre-commercially thinned or fertilized five years prior to establishment.

Foliage and soil samples were collected in the spring following plot installation. The study area was subdivided into four longitudinal panels and six latitudinal bands (Fig. 2.1). Sample plots were systematically located within the panels and bands. Dominant forest types within the sampling region are Douglas-fir and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) with a narrow zone of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and western hemlock along the coast (Franklin and Dyness 1973). The geographical setting includes the coastal portion of the Klamath-Siskiyou Mountains, the Oregon Coast Range, and the Willapa Hills in SW Washington. Precipitation and temperature vary across the region due to elevation, latitude, and the rain shadow on the eastern slope of the Oregon Coast Range. Annual precipitation ranges from 1200-4800 mm, and occurs primarily from October- May. Mean annual temperature ranges from 13-18°C. Elevation of research plots ranges from 40-800m.

The Oregon and Washington Coast Ranges are comprised of ancient and subsequently uplifted oceanic deposits. Soils in the region are formed from a range of parent materials dominated by sediments (mostly sandstone and silt from the Tyee and Yamhill formations), igneous rocks (mostly basalt from Siletz River and Tillamook volcanics), and minor components of meta-sedimentary rocks and gabbro. Soils are variable with Andisol, Inceptisol, and Ultisol soil orders represented. Soil concentrations of organic material and total nitrogen concentrations tend to be higher in coastal areas than in inland areas (Weissenborn 1969).

Field Methods

To sample SNC severity and nutrient content of foliage, we climbed 5-10 codominant trees in each plot and collected samples of branches at 3 different canopy heights (upper, mid, and lower) on the south side of the tree crown. Foliage samples were transported to the lab and stored in a 5˚C cold room. Needle retention was determined by estimating the number of years (annual cohorts) of foliage present on a 4-year-old or older stem (Maguire et al. 2011). Branch samples from all three levels in the crown were used to evaluate relationships between SNC disease severity and needle retention. Only mid-crown branches were used for evaluation of SNC severity and foliar nutrient statistics.

SNC Disease Severity Index

For each canopy height level, 50 individual needles were randomly selected from each 2-year-old branch. Needles were taped on an index card and stored at -20˚C. Needles were then examined under a microscope for evidence of occluded stomates and presence or absence of pseudothecia, which is called “incidence” in this study. The first 10 needles with pseudothecia present were then examined to determine the % of stomates occluded in three regions (base, mid, and tip) of the needle. In each region, we picked a starting point based on a random number and examined 100 stomates from the starting point to determine the number that were occluded by pseudothecia. Pseudothecial occlusion in the three regions was then averaged for each needle and then averaged for 10 needles per tree. The SNC disease severity index was calculated as the ratio of occluded stomates × the percentage of needles with pseudothecia for the 50 needles.

Foliar Analyses

Concentrations of carbon and nine nutrients (N, Na, K, P, Ca, Mg, Mn, Al, S) were measured in one-year-old needles from the mid-crown samples. We chose these nutrients because some of these nutrients would be limiting to tree growth (N, and P), and that others could be important for cell wall stability, solute transport across the cell wall, or general disease resistance (K, Na, Ca, Mg, Mn, Al, and S) (Datnoff et al. 2007). After transporting foliage samples to the lab, approximately 200 1-year-old needles were randomly selected from the mid-crown samples of 5-10 trees from each plot, and dried for 48 hours in a drying oven at 40˚C. The dried samples when then ground with a ball grinder and stored in combustion vials.

Total C and N were determined on dried and ground foliar material using dry combustion on a Thermo FlashEA 1112 (Thermo Fisher Scientific Inc. USA). Foliar samples were digested using 30% H_2O_2 and a 1:10 nitric-hydrochloric (HNO_3-HCL) acid digestion in conjunction with external heating (EPA method 3050; Benton and Wolf, 1997). Digests and extracts were analyzed for K, Na, Ca, Mg, Mn, Al, P, and S with inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Thermo Scientific ICP-OES 61E.

Data Analysis

Before evaluating relationships between nutrient concentrations and the SNC disease severity index, we first investigated whether the severity index was associated with needle retention and whether we should use samples from one level or all levels in each tree to assess relationships. We used R (v. 3.4.3, R Core Team 2017) packages dplyr (Wickham et al. 2017), ggplot2 (Wickham 2009), and nlme (Pinheirro, et al. 2017) to test and plot the effects of canopy height level on the mean average SNC disease severity index of 106 plots with a linear mixed model. Canopy levels and needle retention were treated as fixed effects, including the interaction term. We used latitude, longitude and plots as random effects, plot was nested under latitude and longitude. Data from three canopy levels varied. In order to loosen the assumptions of equal variance, we used Bayesian information criterion (BIC) to select the best model from a set of six a priori models (Table 2.1). Based on the lowest BIC value, we selected the linear mixed model with general correlation adjustment to allow different variations with each canopy level. Samples from the mid-crown had the strongest relationship between mean SNC disease severity index and needle retention. Therefore, only mid-crown data were used in analyzing the relationship between mean SNC disease severity index and individual foliage nutrients. Nutrient concentration was treated as the fixed effect, and latitude and longitude were treated as random effects. Individual nutrients were tested ignoring latitude, longitude, and interaction effects, which assumed all 106 plots were independent.

After testing the individual nutrients across all plots, the latitude/longitude effects were considered as another fixed effect separately in the relationship between mean SNC disease severity index and nutrient concentration. Plots nested under longitude/latitude were included in random effects. There were no interactions between nutrients, latitude and longitude had been tested but not significant, so they are ignored in the final models.

Results

Canopy effects on association of SNC disease severity index and needle retention

There was moderate evidence that relationships between mean SNC disease severity index and needle retention differed among canopy levels (Fig. 2.2; $F_{2, 207}$=5.4, p-value=0.005). The strongest relationship between mean SNC disease severity index and needle retention was in the mid-canopy layer (Fig. 2.2). For a 1-unit increase in needle retention, mean SNC disease severity index was estimated to decrease 2.51 units more in the middle crown than in the lower crown ($t_{2, 207}$=-3.1, p=0.002). There was no evidence that the SNC disease severity index and needle retention were different in the upper canopy and lower canopy ($t_{2, 207}$=-0.6, p=0.53), and the relationship between SNC disease severity index and needle retention did not differ between the upper canopy and mid-canopy ($t_{2, 207}$=1.46, p=0.14).

Needle retention and SNC disease severity in the mid-crown

Based on samples collected from the mid-crown layer we found strong evidence that needle retention was negatively associated with mean SNC disease severity index

($F_{1, 83}$= 23.9, p<0.001, Fig. 2.3). The modeled relationship indicated that needle retention increased by 1 year for every 6.27 decrease in the mean SNC disease severity index (95% C.I. = 4.99-7.55 decrease, $t_{1, 83}$= -4.9, p<0.001).

Foliage nutrients and SNC disease severity in the mid-crown

Of the ten nutrients examined in first-year needles, five (total N, total C, Na, K, and S) had statistically significant relationships with mean SNC disease severity index (Table 2.3). Concentrations of Ca, P, Mg, Mn, and Al in first-year needles did not show statistically significant association with mean SNC disease severity index (Table 2.3).

Interactions between mid-crown foliage nutrients, location and SNC disease severity

We found only limited evidence that relationships between nutrients and SNC disease severity index differed based on latitude or longitude (Table 2.4). The nutrients that differed with latitude were Mn and Al; N, Na, Ca, and Mg differed with longitude (Table 2.4).

Discussion

Nutrient association

The research examining relationships between foliage diseases and foliage nutrients in conifers is poorly understood, and much of the information regards response to fertilization (Danoff et al. 2007). We studied unfertilized plantations across a geographic and climate gradient, and found that higher foliar N was positively associated with the SNC severity index. The linkage between foliar N and SNC susceptibility has been suggested since the beginning of the outbreak in the Pacific Northwest (Waring et al. 2000). El-Hajj et al. (2004) hypothesized that *N. gaeumannii* might acquire nitrogen and carbon from apoplastic spaces of Douglas-fir needles. By using isotopes they found that a high percentage of nitrogen in fungal pseudothecia was from the host needles. In contrast, the relationship between carbon in pseudothecia and infected needles was weak. Perakis et al. (2006) suggested that higher soil N and lower soil Ca were associated with severe SNC disease. Our results were in partial agreement with Perakis (2006) in that we found higher foliar N was positively associated with the SNC severity index. However, we found no evidence that the SNC severity index was associated with the concentration of foliar Ca.

One possible explanation for the positive relationship between nitrogen concentration in one-year-old needles and SNC severity could be that severe SNC infection on two-year-needles causes translocation of N to one-year-needles. Nitrogen in plants is highly mobile and usually concentrated in active tissues (Pallardy, 2008). Severe SNC infection might cause heavy defoliation on the second year needles, thereby causing nitrogen translocation from older needles to juvenile needles. Thus, the tree may be actively moving N to one-year old-needles as two-year old needles die and fall off. Further investigation is needed to confirm this hypothesis.

In our study, both total N and total C concentration in foliage were associated with SNC severity. We believe that there are some relationships between SNC, foliage N, and foliage C. Foliar carbon concentration is commonly related to foliar nitrogen concentration because carbohydrates are products of photosynthesis. Saffell et al. (2014) found that foliar concentrations of non-structural carbohydrates (NSC) were unrelated to SNC disease severity. However, Saffell et al. (2014) also suggested that severely infected trees had less NSC in the trunk than trees with less severe infections.

Concentrations of N, Na, K, S, and C in one-year-old needles were significantly associated with SNC disease severity index on two-year-old needles in coastal Oregon and SW Washington. However, studies about foliage disease and foliage nutrients are rare (Table 2.5). More studies are needed for interpret the cause-effect. This type of analysis provides a hypothesis testing framework and does not attempt to build a model to explain landscape variation and nutrient interactions.

Nutrient concentration in soil and foliage can be changed by fertilization, which was considered to contribute to SNC severity. Mulvey el al. (2013) investigated fertilization response with a variety of applications that included two types of Ca application, P, and custom fertilizer blends (with Mg and Mn) and did not see a response in SNC disease severity with any treatment. Although management recommendations for Douglas-fir forests impacted by SNC have discouraged the use of N fertilizer, Mulvey et al. (2013) found that increased soil and foliar N in a fertilization experiment showed no evidence of a disease severity response.

Nutrient associations by latitude and longitude

Several previous studies have found that elevation and distance from the ocean are important factors related to SNC disease severity in the Oregon Coast Range (Shaw et al. 2014, Lee et al. 2013). Lee et al. (2013) suggested that the SNC severity was higher at lower elevations and that the negative effects of SNC on tree growth were more pronounced at lower elevation areas closer to the coast. We did not see this longitudinal trend in our study. However, latitude played an important role on SNC disease severity in our study. SNC was more severe in the northern study plots than in the southern plots. Climate factors could be causing this difference, since it tends to be warmer and drier in southern Oregon than in northern Oregon. Also, on the southern Oregon coast, the vegetation differs from the north Coast, gradually changing from forests dominated by Douglas-fir in the north to forests of Port-Orford-Cedar (*Chamaecyparis lawsoniana* (A. Murray) Parl.), western hemlock, and coastal redwood (*Sequoia sempervirens* (D.Don) Endl.) in the south (Franklin and Dyness, 1973). These changes in co-dominant vegetation could be another reason that we found less SNC disease severity in the southern Oregon Coast Range. In our study, we had fewer plots in southern Oregon because it was difficult to find plots meeting our target criteria for the SNC monitoring network.

Needle retention and disease severity

In our study, needle retention in the mid-crown layer was more negatively associated with SNC disease severity index than samples collected in the lower and upper crown layers. This was an important finding because needle retention has been used in growth and yield models to estimate the impacts of SNC on volume growth of trees (Maguire et al. 2002, 2011). Shaw et al. (2014) found lowest retention in the upper crown and highest retention in the lower crown across 76 permanent plots in the northern Oregon Coast Range. Although Shaw et al. (2014) found that needle retention was higher in the lower canopy, it was not highly associated with SNC severity in our study. Our anecdotal observations suggest that needles in lower canopy position might be affected by other pathogen such as *Rhizoctonia* and *Phytophthora* (Buhl, et al. 2016), or needle mortality due to limited light, resulting in poor association between SNC and needle retention in the lower canopy. In the upper crown of young trees, there are no four-year old lateral branches. As a result, we recommend the use of mid-crown data for analyses of SNC severity in young Douglas-fir plantations.

Conclusions

In this paper, the scope of inference was limited to Douglas-fir plantations in Oregon and SW Washington coastal regions. Although there are associations between foliage nutrients (N, K, Na, S) and disease severity, we have yet to show cause-effect, which would require experimental treatments. We suggest that mid-crown needles are best for investigations of relationships between SNC severity and environmental covariates such as nutrients, precipitation, and geographic location. Relationships between foliage nutrients and *N. gaeumannii* are still unknown, but our study narrowed down the potential nutrients related to SNC disease severity for further consideration of *N. gaeumannii* infection ecology. It is important to consider that foliar fungi could be influenced by foliar nutrients.

Acknowledgements

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Figure 2.1. Distribution of 106 research plots established in 2013-2015 by the Swiss Needle Cast Cooperative (SNCC) in western Oregon and southwestern Washington (Ritóková et al. 2017).

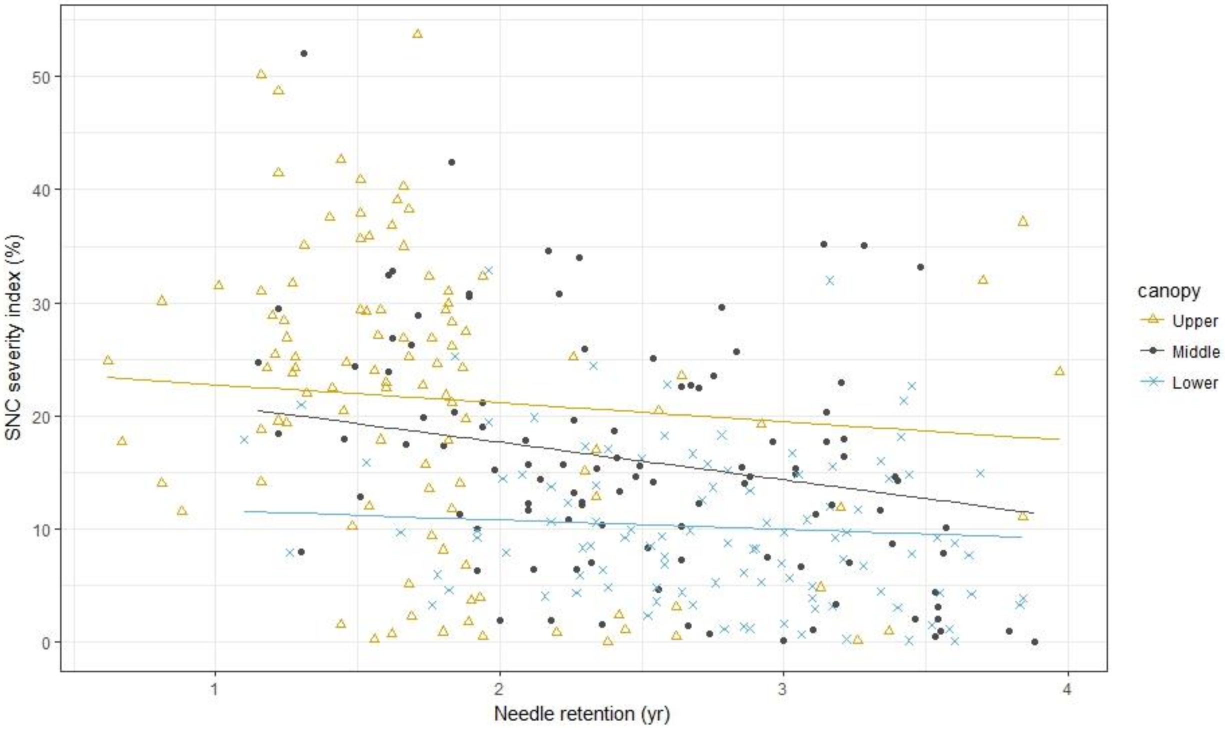

Figure 2.2. Estimated relationships between mean Swiss needle cast disease severity index and needle retention at three canopy levels in young Douglas-fir plantations in western Oregon southwestern Washington, 2013-2015 Regression lines represent samples from the upper, middle, and lower canopy from top to bottom respectively.

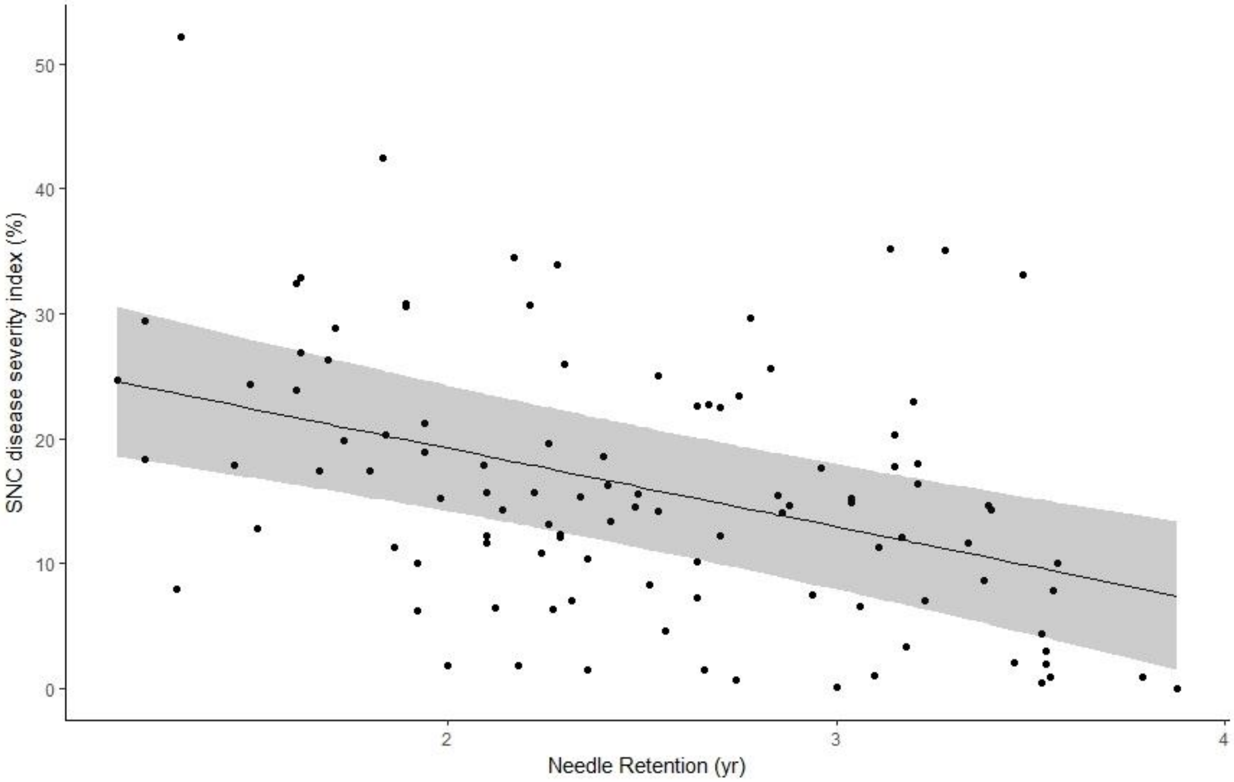

Figure 2.3. Estimated relationship between mean SNC disease severity index and needle retention at mid-crown level in young Douglas-fir plantations in western Oregon southwestern Washington, 2013-2015. Shaded area indicates 95% confidence interval.

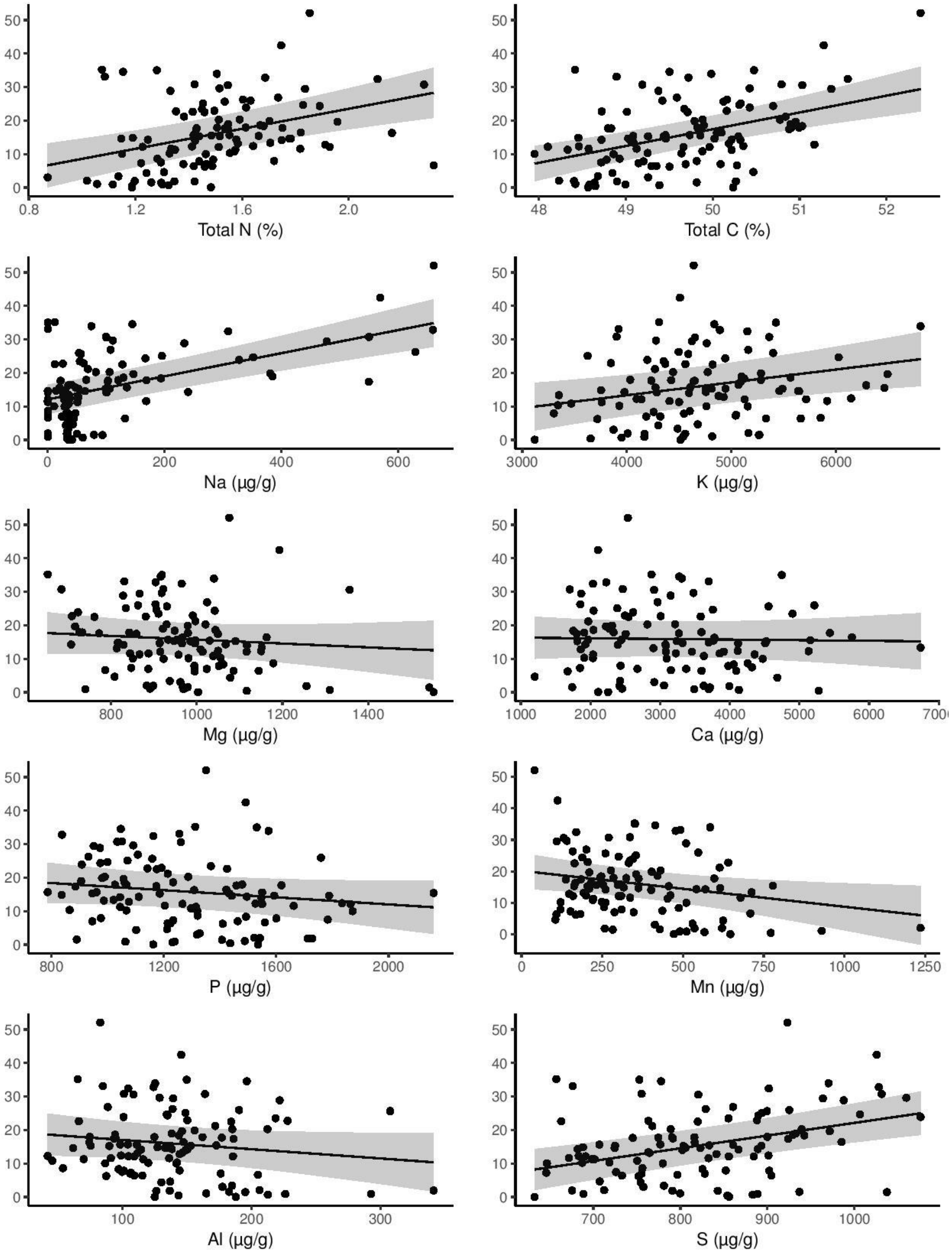

Figure 2.4. Estimated relationships between mean SNC severity index and foliage nutrients at mid-crown level in young Douglas-fir plantations in western Oregon southwestern Washington, 2013-2015. Shaded areas indicate 95% confidence intervals. Y-axis is SNC disease severity index (%). Significant positive relationships were found for N, carbon, Na, K, and S (p<0.01).

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Table 2.1. Model comparisons of loosening equal variance assumption of linear mixed model, to allow the difference variance of each canopy level. Data based on 106 research plots established by the Swiss Needle Cast Cooperative in western Oregon and southwestern Washington in 2013-2015.

Error, variance, and correlation structure for the linear severity model	df	AIC	BIC	logLik
General correlation structure	**13**	**2150.03**	**2198.69**	**-1062**
Autogressive lag 1	11	2179.89	2221.07	-1078.9
Initial model	10	2208.65	2246.08	-1094.3
Allowing for variance heterogeneity	10	2208.65	2246.08	-1094.3
Compound symmetry model	11	2210.65	2251.82	-1094.3
Allowing for both variance heterogeneity and correlation	11	2210.65	2251.82	-1094.3

Table 2.2. Summary of measurements at 106 research plots established by the Swiss Needle Cast Cooperative in western Oregon and southwestern Washington in 2013-2015.

	Average	Standard deviation	Unit
SNC disease severity index	15.87	8.09	%
Needle retention	2.51	0.56	year
Total foliar C concentration	49.67	0.71	%
Total foliar N concentration	1.50	0.20	%
Foliar Na concentration	108.20	100.96	µg/g
Foliar K concentration	4704.26	563.60	µg/g
Foliar Ca concentration	3158.13	873.79	µg/g
Foliar P concentration	1274.62	228.24	µg/g
Foliar Mg concentration	963.27	111.52	µg/g
Foliar Mn concentration	360.10	156.93	µg/g
Foliar Al concentration	138.71	38.54	µg/g
Foliar S concentration	823.41	86.30	µg/g

Table 2.3. Associations between nutrients and SNC disease severity index in 106 plots in coastal Oregon and southwestern Washington, 2013-2015. Test results were based on linear mixed model.

Nutrient	$F_{1, 83}$	p-value		Est. Intercept	Est. Slope	SE
C	25.6	< **0.001**	***	-232.69	5.00	0.99
N	20.4	< **0.001**	***	-6.34	14.94	3.31
Na	44.0	< **0.001**	***	12.12	0.03	0.01
K	8.7	**0.004**	**	-2.01	0.00	0.00
Ca	0.0	0.84		16.50	-0.00	0.00
P	2.3	0.13		22.67	-0.01	0.00
Mg	0.8	0.36		21.41	-0.01	0.01
Mn	5.7	**0.02**		20.15	-0.01	0.00
Al	2.5	0.12		19.83	-0.03	0.02
S	20.8	< **0.001**	***	-15.76	0.04	0.01

Table 2.4. Statistical significance of interaction with latitude and longitude between SNC disease severity index and nutrients in 106 plots in coastal Oregon and southwestern Washington, 2013-2015. If the interaction effect is statistically significant, that means the relationship between SNC disease severity index and nutrient differs by latitude or longitude.

	Nutrient effect			Latitude effect			Nutrient: Latitude effect		
	$F_{1, 78}$	p-value		$F_{5, 16}$	p-value		$F_{5, 78}$	p-value	
C	35.5	< **0.001**	***	3.7	**0.021**	*	0.7	0.635	
N	19.9	< **0.001**	***	8.0	**0.001**	***	1.5	0.192	
Na	58.0	< **0.001**	***	5.1	**0.006**	***	1.4	0.218	
K	3.7	0.057		7.2	**0.010**	***	0.1	0.982	
P	4.5	**0.036**	*	4.9	**0.007**	***	0.7	0.651	
Ca	0.2	0.645		4.1	**0.015**	**	0.6	0.678	
Mg	5.2	**0.025**	*	4.2	**0.013**	**	1.1	0.364	
Mn	12.1	**0.001**	***	4.1	**0.014**	**	2.6	**0.032**	*
Al	2.9	0.094		6.0	**0.003**	***	3.3	**0.010**	**
S	20.2	< **0.001**	***	8.0	**0.001**	***	1.3	0.283	
	Nutrient effect			Longitude effect			Nutrient: Longitude effect		
	$F_{1, 80}$	p-value		$F_{3, 18}$	p-value		$F_{3, 80}$	p-value	
C	28.7	< **0.001**	***	0.7	0.559		0.5	0.670	
N	14.2	< **0.001**	***	0.1	0.962		2.4	**0.077**	*
Na	50.0	< **0.001**	***	1.9	0.164		4.8	**0.004**	***
K	4.1	**0.047**	**	0.4	0.747		0.4	0.736	
P	2.0	0.161		0.4	0.736		1.3	0.287	
Ca	0.0	0.987		0.7	0.576		2.9	**0.040**	**
Mg	2.1	0.147		0.7	0.547		2.8	**0.045**	**
Mn	7.4	**0.008**	***	0.4	0.740		1.2	0.318	
Al	2.0	0.158		0.6	0.656		2.0	0.121	
S	12.8	**0.001**	***	0.2	0.912		1.4	0.238	

Table 2.5. Summary of literature on relationships between conifer foliage diseases and key foliage nutrients within the leaf, including results from our study.

Nutrient	Effect	Citation	Our study
N	Generally thought to increase disease but not always	Conifer literature rare. Mulvey et al. 2013: no impacts of fertilization SNC. El Hajj et al. 2004: yes there was an impact on SNC from fertilization. Lambert 1986: Dothistroma infection increased in N fertilized plot	Positive
C	Correlated with N		Positive
	Foliar NSC retains high in infected needles	Saffell et al. 2014	
K	Pinus needle shedding, K decreases in shedding needle	Bruning 1965, cited in Prabhu et al. 2007	Positive
	Pinus strobu rust (*Peridermium* spp.), K decreases in disease	Hutchinson 1935, cited in Prabhu et al. 2007	
Na	Conifer foliage disease relationships unknown		Positive
Ca	No effect in fertilization and SNC	Mulvey et al. 2013	None
	Conifer foliage disease relationships unknown		
P	Fusiform rust on pine, P increases in disease	Prabhu et al. 2007	None
	No response to fertilization and SNC	Mulvey et al. 2013	
Mg	Included in custom fertilizer blends and no response	Mulvey et al. 2013	None
	Conifer foliage disease relationships unknown		
Mn	Included in custom fertilizer blends and no response	Mulvey et al. 2013	None
	Conifer foliage disease relationships unknown		
Al	Conifer foliage disease relationships unknown		None
S	Conifer foliage disease relationships unknown		Positive

CHAPTER 3 - ASSOCIATIONS BETWEEN SWISS NEEDLE CAST SEVERITY AND CLIMATE FACTORS IN YOUNG-GROWTH DOUGLAS-FIR IN COASTAL WESTERN OREGON AND SOUTHWESTERN WASHINGTON, USA

Abstract

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii*) caused by *Nothophaeocryptopus gaeumannii*, an ascomycete fungus (Mycosphaerellaceae) that causes growth reductions in Douglas-fir plantations in the Pacific Northwest. Epidemiology of the fungus is thought to be associated with climate. Our study was conducted in 106 systematically placed research plots established by the Swiss Needle Cast Cooperative in 2013-2015. Climate variables tested were monthly and annual precipitation, minimum temperature, maximum temperature, mean temperature, mean dew point temperature, and maximum VPD. We also examined the influence of latitude and longitude on climate variables and severity of SNC. Minimum temperature and dew point temperature were the most significant factors related to SNC disease severity ($p<0.001$). Oct-Apr mean temperature, Oct-Apr maximum temperature, and Nov-Apr maximum VPD were also associated with SNC disease severity ($p<0.001$). Monthly precipitation was not associated with mean SNC disease severity during the summer months. Dew point temperature for all months was positively associated with SNC disease severity ($p<0.001$). We suggest that dew point temperature may be more important in epidemiology of *N. gaeumannii* than previously thought. Latitude had a strong relationship with SNC disease severity and climate variables, while longitude did not. Analyses of climate relationships within subregions in the study area indicated that relationships between SNC disease severity and climate variables were strongest in the Tillamook region of northwest Oregon.

Introduction

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) caused by the fungus, *Nothophaeocryptopus gaeumannii* (T. Rohde) Videira et al. (Ascomycete: Mycosphaerellaceae) (Videira et al. 2017). In coastal Oregon and Washington, it causes growth loss in Douglas-fir plantations (Shaw et al. 2011). Historically, the disease was thought to be unimportant in forest plantations, but in the 1990's SNC emerged as a major influence on tree growth, and is now seen as a significant threat to Douglas-fir (Hansen et al. 2000). Aerial surveys in 1996 detected 53,050 ha of forest in the Oregon Coast Range with symptoms of SNC. A resurvey of the same region in 2015 detected 238,705 ha of forest with symptoms of SNC (Ritóková et al. 2016). Although SNC generally does not cause mortality, annual wood volume losses were estimated at 23% in the epidemic area, with some plantations experiencing up to 52% growth losses (Maguire et al. 2002). Although it appeared that the impacts of SNC varied with geographical setting, climate, and plantation attributes, relatively few data were available to test these hypotheses.

Nothophaeocryptopus gaeumannii is a native endophytic fungus specific to Douglas-fir, and only causes visible disease when the stomates are occluded by fungal reproductive structures (pseudothecia). Light infections may not be noticeable, but if over 50% of the needle stomates become plugged by pseudothecia, infected needles fall from the tree (Hansen et al. 2000). Overall needle retention of less than 3 years may cause reduced growth, as well as visible symptoms of chlorosis (Maguire et al. 2011). Because infection rates of 2-year-old needles tend to be correlated with disease effect, disease severity of SNC is usually determined by examination of 2-year-old needles (Manter et al. 2005).

Studies by Rosso and Hansen (2003) and Manter et al. (2005) indicated that warmer temperatures and higher moisture had a positive influence on the abundance of *N. gaeumannii*. The seasonal temperature, especially winter temperature, may be related to rapid development of the fungus within the needle. Additionally, late spring and summer temperature can affect spore germination and initial colonization on needles. Leaf wetness, caused by precipitation and atmospheric conditions during spore dispersal (May – August) is crucial for fungal colonization of needles. In particular, precipitation in June and July is positively correlated with intensification of *N. gaeumannii* (Rosso and Hansen 2003).

Based on an analysis of growth rings in mature/old-growth Douglas-fir, Lee et al. (2017) suggested that warming temperatures might increase the impact of SNC disease on tree growth at low to mid elevations on the west slope of Cascade Mountains in Oregon. *N. gaeumannii* has also been introduced to New Zealand, where it exhibits responses to temperature and precipitation that are similar to responses observed in coastal Oregon and SW Washington. Disease severity of SNC in New Zealand was positively associated with winter temperature (Stone et al. 2007, Watt et al. 2010) and spring precipitation (Watt et al. 2010).

Although there have been numerous studies in which researchers have investigated relationships between SNC and climate variables, most have been based on limited geographic coverage and have often used different definitions of seasonal periods.

This makes comparisons among studies difficult. In this study, we examined relationships between SNC severity and a variety of climate variables in an extensive network of research plots established by the Swiss Needle Cast Cooperative (SNCC) in western Oregon and southwest Washington in 2013-2015 (Ritóková et al. 2017). The sampling frame consisted of a network of 106 plots that were systematically placed in 10-30-year-old Douglas-fir plantations within 56 km (35 miles) of the Pacific Ocean from the California border to southwest Washington (Fig. 2.1).

In this study, we used the research and monitoring plot network established by the Swiss Needle Cast Cooperative (SNCC). We examined associations between SNC severity and a suite of monthly weather variables, including precipitation, minimum temperature, maximum temperature, mean temperature, mean dew point temperature, and maximum vapor pressure deficit (VPD). We also examined associations between latitude and longitude and SNC disease severity. The primary question of interest was "Are specific weather variables associated with disease severity of *N. gaeumannii* in Douglas-fir?" We hypothesized that late spring and early summer precipitation and winter mean temperature would be statistically significant factors associated with SNC disease severity index.

Methods

Study Sites

The 106 SNCC study plots were installed during 2013-2015, including 98 plots in western Oregon and 8 plots in southwestern Washington (Fig. 2.1). Each plot consisted of a 1/5-acre (0.08 ha) of uniform-age trees between 10-25 years old. The targeted basal area composition was 80% Douglas-fir and 300-400 trees per acre. All trees were tagged and measured for diameter at 137 cm, and a subset of 40 trees were measured for height. All plots were established in plantations that had not been pre-commercially thinned or fertilized five years prior to establishment.

Foliage and soil samples were collected in the spring following plot installation. The study area was subdivided into four longitude panels and six latitude bands (Fig. 2.1). Sample plots were systematically located within the panels and bands. Dominant forest types within the sampling region were forests of Douglas-fir and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) with a narrow zone of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and western hemlock along the coast (Franklin and Dyness, 1973). The geographical setting included the coastal portion of the Klamath-Siskiyou Mountains, the Oregon Coast Range, and the Willapa Hills in SW Washington. Precipitation and temperature varied across the region due to elevation, latitude, and the rain shadow on the eastern slope of the Oregon Coast Range. Annual precipitation ranged from 1200-4800 mm, and occurred primarily from October- May. Mean annual temperature ranged from 13-18°C. Elevation of research plots ranged from 40-800m.

The Oregon and Washington Coast Ranges are made up of ancient and subsequently uplifted oceanic deposits. Soils in the region are formed from a range of parent materials dominated by sediments (mostly sandstone and silt from the Tyee and Yamhill formations), igneous rocks (mostly basalt from Siletz River and Tillamook volcanics), and minor components of meta-sedimentary rocks and gabbro. Soils are variable with Andisol, Inceptisol, and Ultisol soil orders represented. Soil concentrations of organic material and total nitrogen concentrations tend to be higher in coastal areas than in inland (Weissenborn 1969).

Field Methods

To sample SNC severity and nutrient content of foliage, a climber collected branch samples of 5-10 codominant trees in each plot at middle canopy on the south side of the tree. Foliage samples were transported to the lab and stored in a 5˚C cold room.

SNC Disease Severity Index

For middle canopy in each tree, 50 individual needles were randomly selected from each 2-year-old branch. Needles were taped on an index card and stored at -20 ˚C. Needles were then examined under a microscope for evidence of occluded stomates and presence or absence of pseudothecia, which is called “incidence” in this study. The first 10 needles with pseudothecia present were then examined to determine the % of stomates occluded in three regions (base, mid, and tip) of the needle. In each region, we picked a starting point based on a random number’s table and examined 100 stomates from the starting point to determine the number that were occluded by pseudothecia. Pseudothecial occlusion in the three regions was then averaged for each needle and then averaged for 10 needles per tree. The SNC disease severity index was calculated as the ratio of occluded stomates × the percentage of needles with pseudothecia for the 50 needles.

Weather Data

Monthly and annual weather data for each plot were downloaded from PRISM map at Oregon State University (http://www.prism.oregonstate.edu/explorer/). Spatial resolution of the map was 800m. Weather variables included precipitation, minimum temperature (Tmin), maximum temperature (Tmax), mean temperature (Tmean), mean dew point temperature (DP), and maximum VPD (VPDmax). Lat-long coordinates of each plot were obtained from a databased maintained by the Swiss Needle Cast Cooperative.

Data Analysis

We used R (v. 3.4.3, R Core Team 2017) and package dplyr (Wickham et al. 2017), ggplot2 (Wickham 2009), and nlme (Pinheirro, et al. 2017) to test and plot relationships between mean SNC disease severity index and individual climate variables of 106 plots with linear mixed model. Monthly climate variable was treated as the fixed effect, and latitude and longitude were treated as random effects. Individual climate variables were tested ignoring latitude, longitude, and interaction effects, which assumed all 106 plots were independent.

After testing the climate variables across all plots, the latitude/ longitude effects were considered as another fixed effect separately in the relationship between mean SNC disease severity index and climate. Plots nested under longitude/latitude were included in random effects. The interactions between climate, latitude and longitude had been tested but not significant, so they are ignored in the final models.

Results

SNC disease severity index and climate variables

Associations between weather variables and mean SNC disease severity index varied among months (Fig. 3.2). The association (estimated slope of fitted model) with precipitation was highest in July, and with larger variation in July and August (the dry months in Pacific Northwest) than other months. Associations with Tmean and VPDmax and DP were higher during the rainy season (Oct-Apr) than during the dry season (May-Aug).

Monthly precipitation was positively associated with the mean SNC severity index in December through March, but not during the rest of the year (Table 3.1). Monthly Tmax and Tmean were statistically significantly associated with the mean SNC disease severity index from Oct –Apr (Table 3.1). Monthly Tmin and DP were statistically significantly associated with the mean SNC disease severity index in every month except for Tmin in Oct (Table 3.1). Monthly VPDmax was significantly associated with the mean SNC disease severity index in Nov-Apr, but not during the rest of the year (Table 3.1). Annual Tmin and annual DP were both associated with the mean SNC disease severity index ($F_{1, 83}$=10.3 and 24.9, p=0.002 and <0.001 respectively), but annual VPDmax was not ($F_{1, 83}$=0.1, p=0.78).

Interactions between climate variables, location and SNC disease severity

Adding interaction terms between weather variables and latitude and longitude indicated that there were associations between several climate variables and latitude

(Table 3.2), but generally no associations between weather variables and longitude (Table 3.3). July precipitation was strongly associated with SNC disease severity index when considering latitude effect as well as the interaction term ($F_{1,78}$=9.4, p=0.003). The only weather variable that had a significant interaction effect with longitude was Aug Tmin ($F_{3, 80}$=3.4, p=0.022).

Latitude was an important factor associated with mean SNC disease severity index and climate variables in this study. There were interactions between latitude and a number of climate variables, including Tmin (Jan-Apr), DP (all months except for Feb, Nov, and Dec), Tmax (May and Sep), Tmean (May and Sep), and VPDmax (Mar, May and Sep) (Table 3.2). Latitude was significant in most analysis when considering monthly climate variables (Table 3.2). Although Tmean in Oct-Apr was strongly associated with mean SNC disease severity index when we ignored the latitude effect (Table 3.1), it was not significant when latitude effect was included in the model (Table 3.2).

When considering differences between latitude grids, the Tillamook area had the strongest associations between mean SNC disease severity index and Tmean, DP, and VPDmax (Fig. 3.6, Fig. 3.7, and Fig. 3.8 respectively). This suggested that relationships between those monthly weather variables and the mean SNC disease severity index were stronger in the Tillamook region than in other regions.

Discussion

There have been many studies in which researchers have examined relationships between climate variables and distribution or severity of SNC disease (Table 3.4).

Although they vary somewhat, the results of these studies and our study suggest that winter temperatures, as well as late spring and summer precipitation and fog, have a positive influence on the severity of SNC. In contrast, summer temperatures appear to have an inverse effect on severity of SNC. Our study also suggests that dew point temperature has a positive effect on SNC severity, particularly in summer (Fig. 3.2). However, there is some evidence that there is not a one-size-fits-all epidemiology for SNC expression (Lee et al. 2013, 2017). Each geographically distinct area in western Oregon and Washington will likely have a unique set of specific drivers. For example, winter temperatures may have a stronger influence on SNC severity in higher elevation areas than in low elevation areas.

Precipitation

Seasonality of precipitation is a major factor that explains risk of infection and epidemics in other conifer foliage fungi (Iturritxa et al. 2015, Woods et al. 2005, 2016, Wyka et al. 2017). Although many previous studies of SNC suggested that spring and/or summer precipitation was positively related to SNC disease severity (Lee et al. 2013, Manter et al. 2005, Rosso and Hansen 2003, Watt el al. 2010, Wilhelmi et al. 2017, Zhao et al. 2012, Table 3.4), we did not find a strong relationship between SNC severity and monthly precipitation during the summer. We concluded that (1) either precipitation does not vary enough to be considered as a driver of disease severity, or (2) leaf wetness is not controlled exclusively by precipitation in the region and other factors may wet leaves such as dew and fog. Because SNC disease is most severe in the upper crown of plantation trees (Hanson et al. 2000, Shaw et al. 2014), it appears that leaf wetness during spore dispersal is not a limiting factor.

Monthly mean temperature and maximum temperature

Winter temperature is considered an important factor associated with SNC disease (Table 3.4) (Lee et al. 2013; Manter et al. 2005; Stone et al. 2007; Stone et al. 2008; Watt et al. 2010; Wilhelmi et al. 2017). Our results also suggest that warmer temperatures during winter and spring (October-April) have a positive influence on SNC disease severity. Lee et al. (2017) predicted that Swiss needle cast disease will spread northward and into higher elevation areas in the future due to warming winters associated with climate change.

Black et al. (2010) found that growth of Douglas-fir was inversely associated with warmer temperatures in March to August in two study areas in western Oregon. Lee et al. (2013, 2017) found that warmer temperatures in June and July were inversely associated with tree growth across a broad region in western Oregon. Rosso and Hansen (2003) found that mean monthly temperature in midsummer (July) was inversely associated with tree growth, and suggested that the observed relationship was due to reduced needle colonization by *N. gaeumannii*. Although it is difficult to resolve some of the variation in these study results, it is possible that the differences are due to differences in elevation and proximity to the coast or to other local conditions.

Dew point temperature

Although summer precipitation was not significantly related to SNC disease severity in this study, dew point temperature was strongly associated with SNC disease severity. Dew point temperature is used for leaf wetness estimation (Mashonjowa et al. 2013), and leaf wetness is known to be a critical variable for successful infection by *N. gaeumannii* (Manter et al. 2005). Dew may be a primary factor that contributes to leaf wetness, in addition to precipitation and fog in Douglas-fir plantations. Lee et al. (2016) considered summer (June-September) dew point deficit (the difference between air temperature and the dew point temperature) to have a negative association with SNC disease severity. We did not model this factor, but it is consistent with dew point temperature being an important positive factor on SNC severity. We also found that monthly minimum temperature was positively associated with SNC disease severity in all months except October. This is likely because Tmin is closely associated with dew point temperature.

Vapor pressure deficit (VPD)

Vapor pressure deficit, the difference between the amount of moisture in the air and the amount possible for the air to hold, is currently considered a major factor in drought related tree mortality due to increased atmospheric moisture demand (Breshears et al. 2013). In our study, maximum VPD was positively associated with SNC disease severity from November through April (Fig. 3.2), which is late fall, winter, and early spring. It might be that higher temperatures that positively influence SNC in this season were associated with maximum VPD. We found that mean temperature and maximum temperature were positively associated with disease severity from October through April. We suggest that high summer VPD would be associated with hotter drought conditions and decreased foliage disease.

Other factors that could influence SNC disease expression associated with climate factors

Seed sources may be another co-factor involved in associations between weather variables and SNC disease severity (Wilhemi et al. 2017). Seed sources used to plant trees in the SNCC research plots came from broad seed zones determined by industry and governments and typically came from genetically improved trees in seed orchards. Recent research has shown that trees produced from seeds in one region may be more susceptible to SNC if transferred to a different region, due local climate adaptation issues (Wilhemi et al. 2017). In addition, the fungus, *N. gaeumannii* has two distinct lineages (Bennett and Stone 2016) that differ geographically, which may also influence disease response.

Climate variable associations by latitude and longitude

Latitude played an important role on SNC disease severity in this study. Generally, SNC infections were more severe in the Coast Range in northern Oregon compared to areas farther south, regardless of weather conditions (Ritóková et al. 2016). Generally, it is warmer and drier in southern Oregon than in the northern Coast Range. Also, the co-dominant tree species change with latitude in Oregon, from forests dominated by Douglas-fir and western hemlock in the north to forests that include Douglas-fir, Port-Orford-Cedar (*Chamaecyparis lawsoniana* (A. Murray) Parl.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), coastal redwood (*Sequoia sempervirens* (D.Don) Endl.) (Franklin and Dyness, 1973) and tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H.Oh) in southwest Oregon. The co-dominant tree species might change the forest structure and affect the local climate, so lack of landscape dominance by Douglas-fir plantations could be another reason that we found less SNC disease severity in south Oregon coast.

The Tillamook area is historically one of the regions in which forests are most severely infected with SNC (Hansen et al. 2000), and in this region even mature forests are often infected (Black et al. 2010). Our results confirm this relationship in that our samples from the Tillamook study plots had stronger associations between disease severity and several of the weather variables examined compared to other regions in our study area.

Rosso and Hansen (2003) suggested that distance from the ocean was inversely associated with SNC severity because atmospheric moisture from the ocean was higher near the coast. In contrast, we found no relationship between distance from the ocean (Longitude) and the SNC severity index. We do not know if increasing our frame of reference to all of western Oregon would change our results, but this certainly needs to be investigated. Also, in our study, we treated distance from the coast as a categorical variable (1 degree intervals) instead of a continuous gradient from the ocean. We do not know if the outcome would be different if distance from the coast was treated as a continuous variable, but we doubt it.

Conclusions

In this paper, the scope of inference was limited to Douglas-fir plantations in Oregon and SW Washington within 56 km from the ocean. Minimum temperature and dew point temperature were the most significant factors related to SNC disease severity. October to April mean temperature, October to April maximum temperature, and November to April maximum VPD were also associated with SNC disease severity. Summer precipitation was not a significant factor in our study but dew point temperature was. Although humidity is important for successful colonization of needles, we suggest that thermal characteristics (i.e. dew point temperature) may have a stronger influence on the development of *N. gaeumannii* and SNC disease severity. Latitude was strongly associated with SNC disease severity and climate variables in our study, while longitude was not strongly associated with climate variables or the SNC severity index.

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Figure 3.1. Distribution of 106 research plots established in 2013-2015 by the Swiss Needle Cast Cooperative (SNCC) in western Oregon and southwestern Washington (Ritóková et al. 2017).

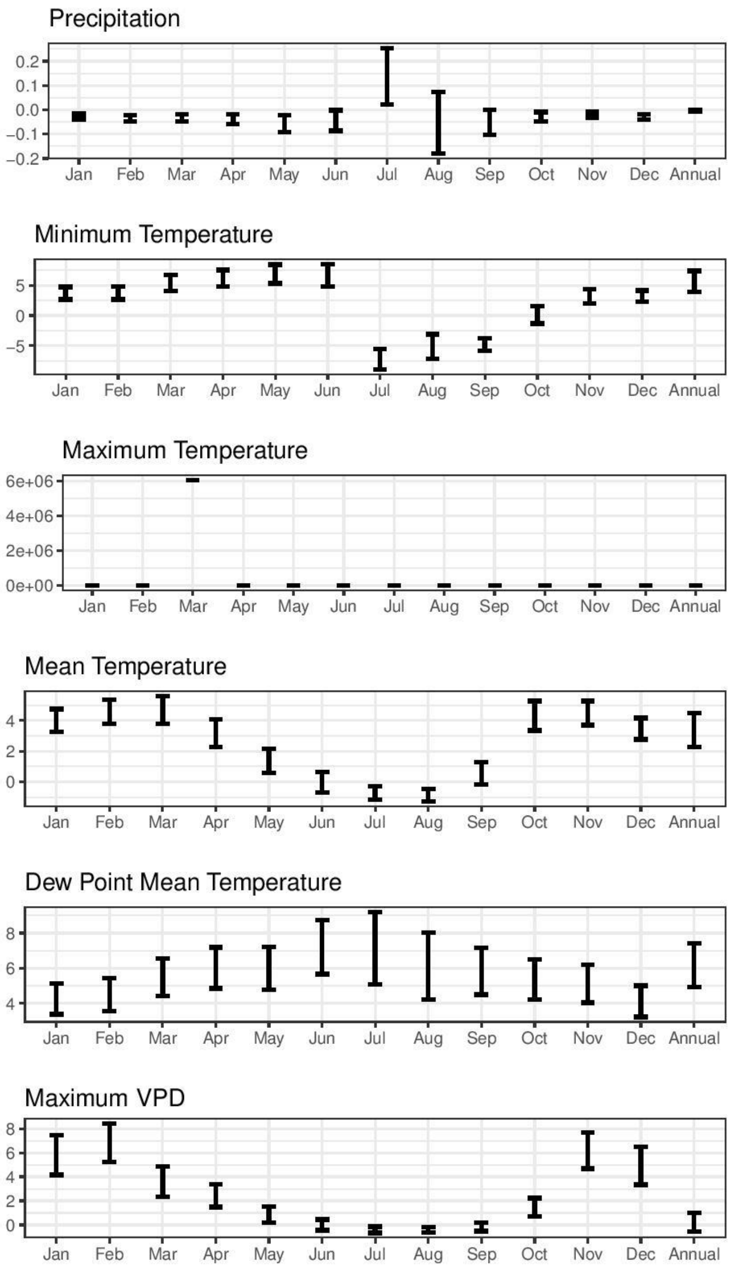

Figure 3.2. Fitted model slopes between SNC disease severity index and climate variables on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. X-axis is month, Y-axis is the range of estimated slope of fitted model. The estimated fitted slope represents the association between SNC disease severity index and monthly climate variables. Notice that the scales are different for each variables based on their fitted models.

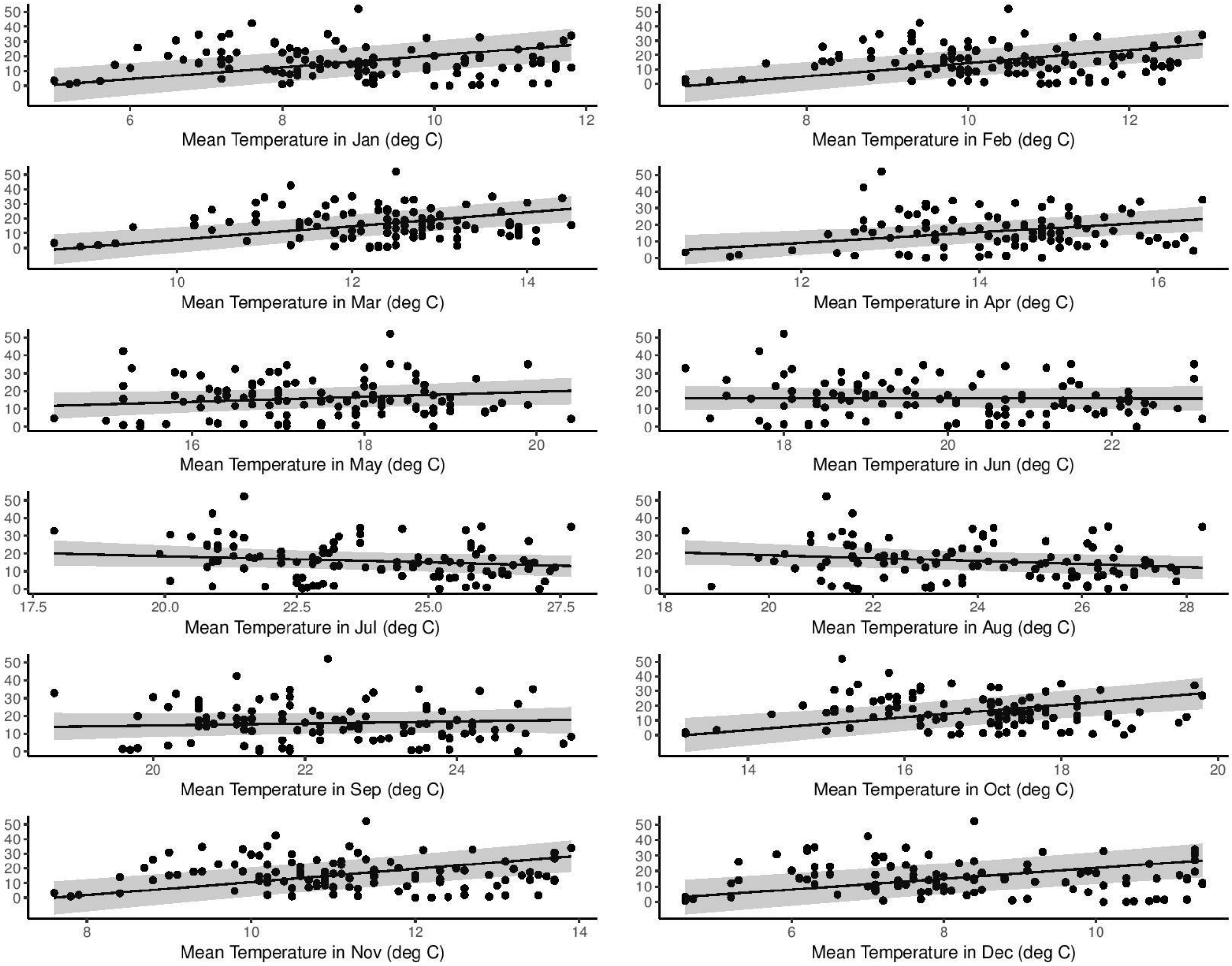

Figure 3.3. Estimated relationships between mean SNC disease severity index and monthly mean temperatures on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. Y-axis is SNC disease severity index (%) and shaded areas indicate 95% confidence intervals. Relationships between SNC disease severity index and mean temperature were statistically significant in Oct, Nov, Dec, Jan, Feb, Mar, and Apr.

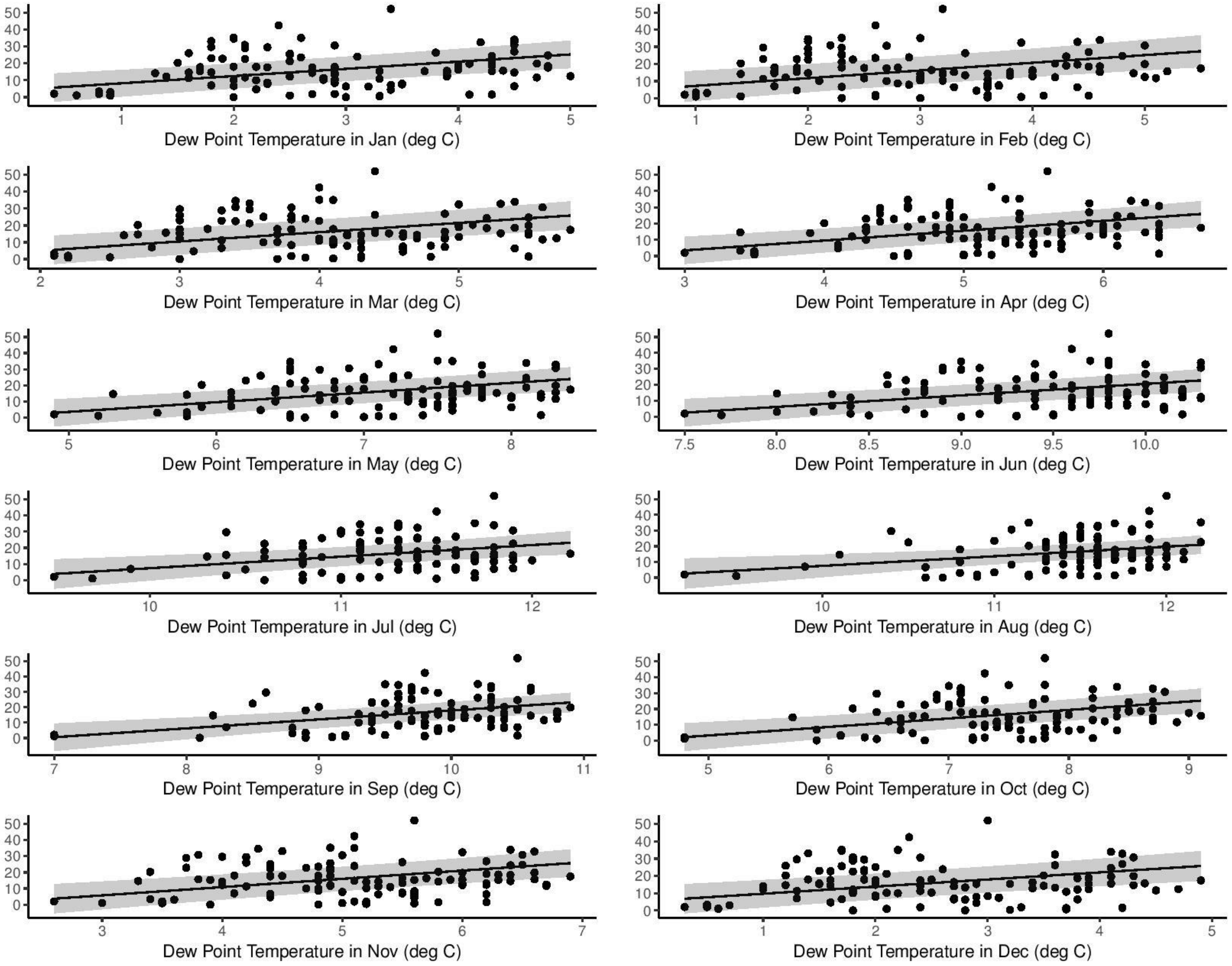

Figure 3.4. Estimated relationships between mean SNC disease severity index and monthly mean dew point temperature on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. Y-axis is SNC disease severity index (%) and shaded areas indicate 95% confidence intervals. Relationships between SNC disease severity index and mean dew point temperature was statistically significant on every single month.

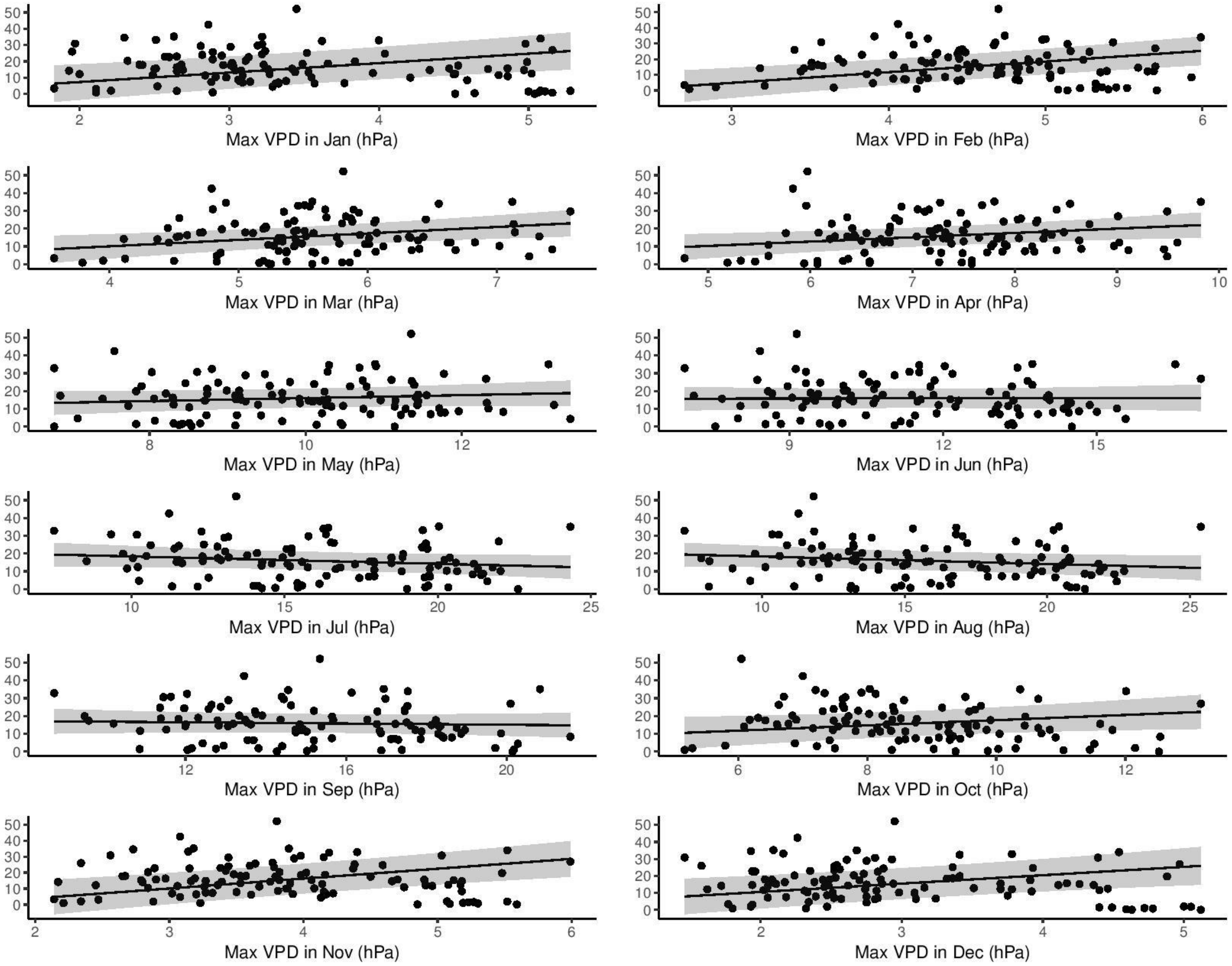

Figure 3.5. Estimated relationships between mean SNC disease severity index and monthly mean maximum VPD on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. Y-axis is SNC disease severity index (%) and shaded area indicate 95% confidence intervals. Relationships between SNC disease severity index and mean maximum VPD was statistically significant on Nov, Dec, Jan, Feb, Mar, and Apr.

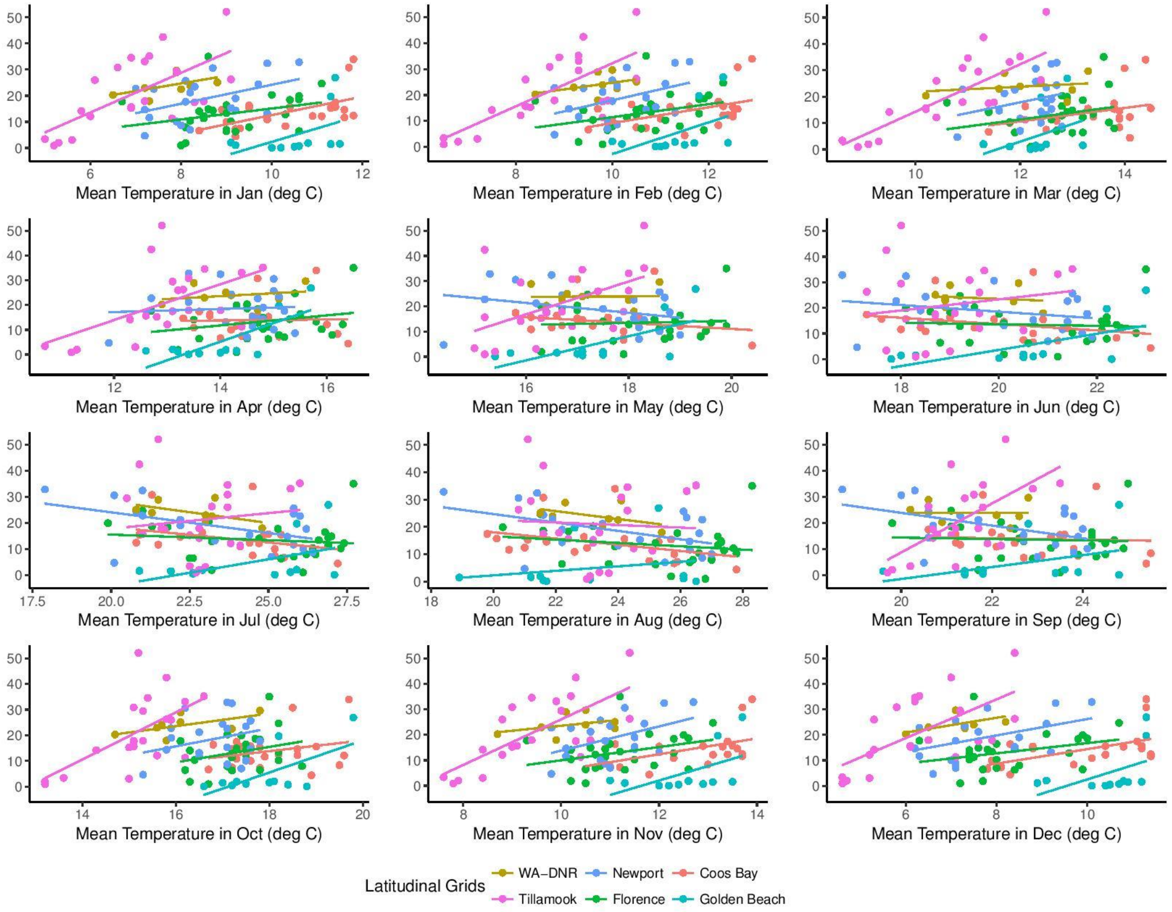

Figure 3.6. Association between SNC disease severity index and monthly mean temperature on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. Y-axis is SNC disease severity index (%). The fitted slope represents the difference of association across latitudes.

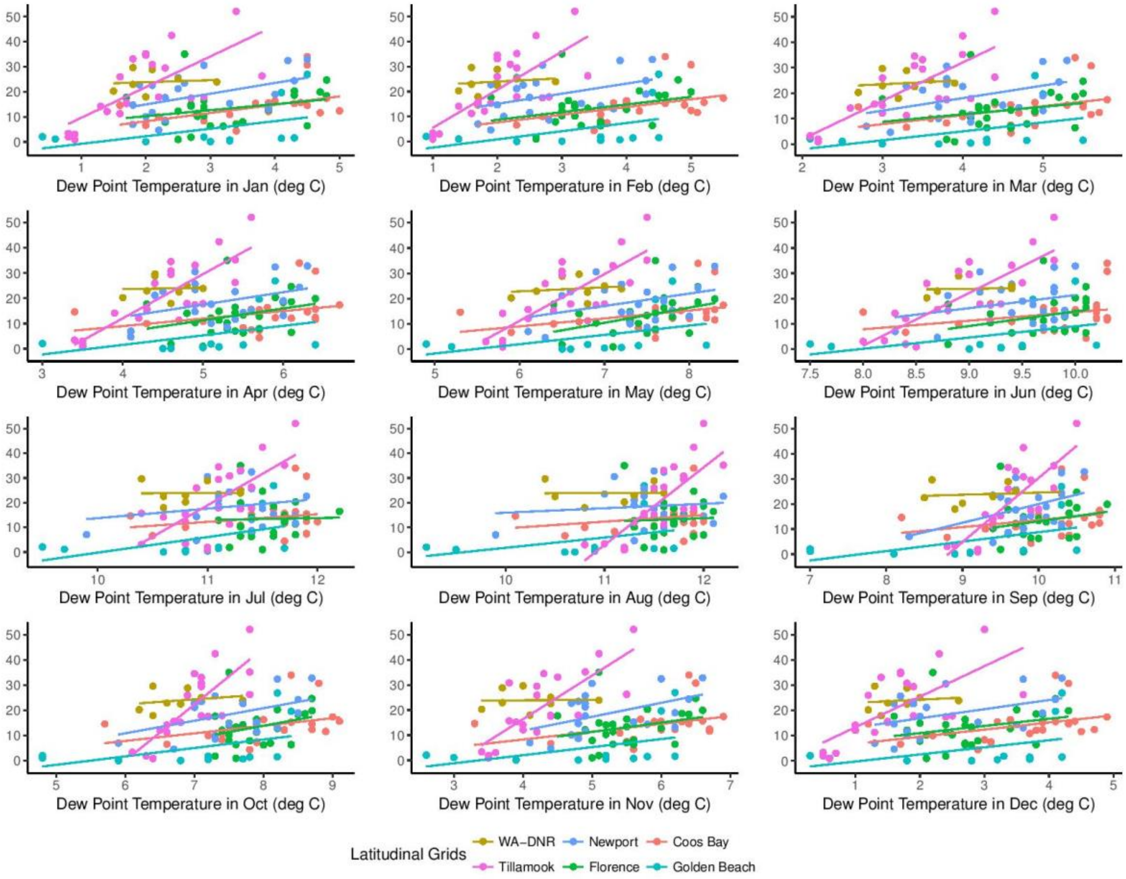

Figure 3.7. Association between SNC disease severity index and monthly dew point mean temperature on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. Y-axis is SNC disease severity index (%).The fitted slope represents the difference of association across latitudes.

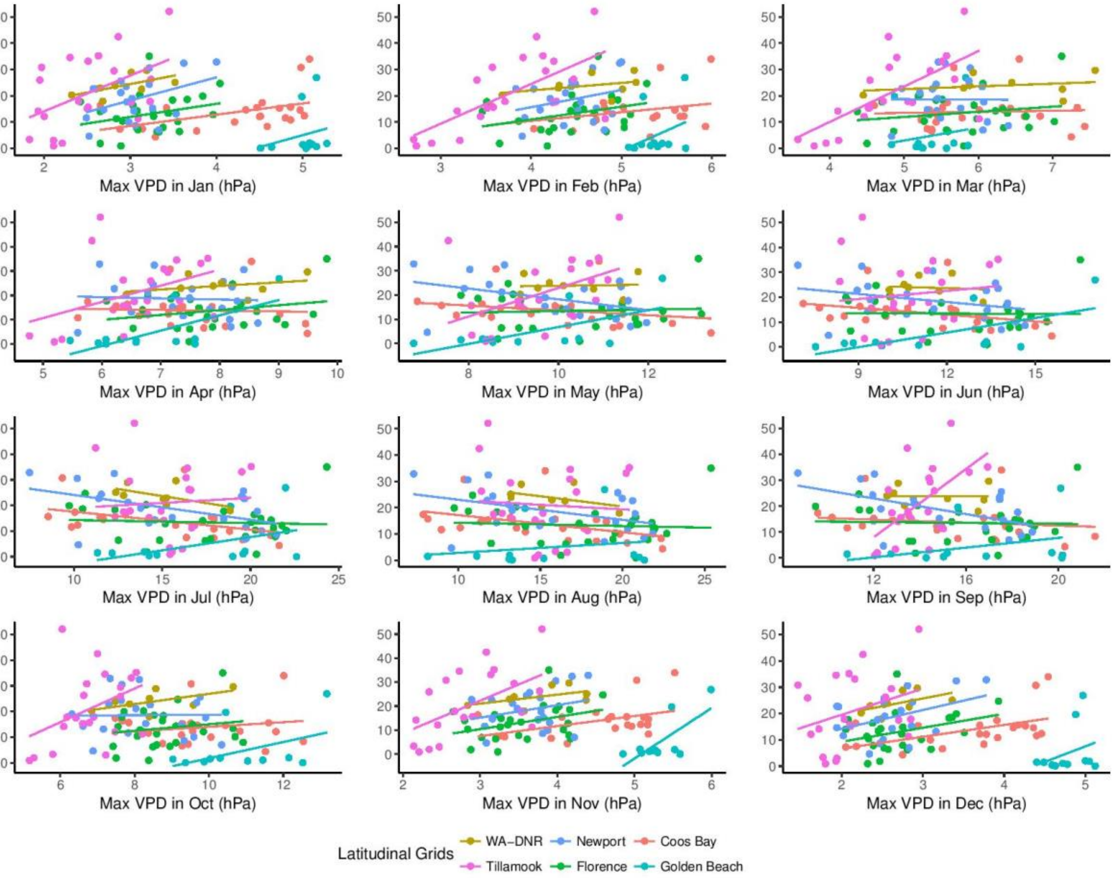

Figure 3.8. Association between SNC disease severity index and monthly maximum VPD on SNCC study plots in coastal western Oregon and southwest Washington in 2013-2015. Y-axis is SNC disease severity index (%).The fitted slope represents the difference of association across latitudes.

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Table 3.1. Results of linear mixed model analyses of associations between weather variables and SNC disease severity index in Douglas-fir plantations in coastal Oregon and southwestern Washington, 2013-2015.

Variables	Month	$F_{1,83}$	p-value		Est. intercept	Est.slope	SE (slope)
Precipt	Jan	6.2	**0.015**	*	25.56	-0.03	0.01
	Feb	6.7	**0.012**	*	25.58	-0.03	0.01
	Mar	5.1	**0.026**	*	24.47	-0.03	0.01
	Apr	3.4	0.069		22.72	-0.04	0.02
	May	2.7	0.102		22.35	-0.06	0.03
	Jun	1.1	0.297		19.35	-0.04	0.04
	Jul	1.4	0.235		12.84	0.14	0.11
	Aug	0.2	0.677		17.36	-0.05	0.13
	Sep	1.0	0.315		19.10	-0.05	0.05
	Oct	2.1	0.155		20.63	-0.03	0.02
	Nov	3.8	0.054		23.19	-0.02	0.01
	Dec	7.5	**0.008**	*	27.27	-0.03	0.01
	Annual	4.6	**0.035**	*	24.22	0.00	0.00
Tmin	Jan	13.2	**0.001**	***	7.45	3.71	1.02
	Feb	12.4	**0.001**	***	7.18	3.76	1.07
	Mar	16.2	**< 0.001**	***	-0.36	5.37	1.33
	Apr	21.5	**< 0.001**	***	-9.14	6.18	1.33
	May	19.0	**< 0.001**	***	-27.98	6.88	1.58
	Jun	12.9	**0.001**	***	-41.30	6.65	1.86
	Jul	18.4	**< 0.001**	***	93.19	-7.25	1.69
	Aug	6.4	**0.014**	*	70.75	-5.11	2.03
	Sep	22.0	**< 0.001**	***	61.62	-4.83	1.03
	Oct	0.0	0.939		15.21	0.11	1.42
	Nov	7.2	**0.009**	*	3.01	3.18	1.18
	Dec	11.4	**0.001**	**	9.40	3.21	0.95
	Annual	10.3	**0.002**	**	-17.24	5.67	1.76
Tmax	Jan	24.3	**< 0.001**	***	-8.77	4.43	0.90
	Feb	30.6	**< 0.001**	***	-17.99	5.35	0.97
	Mar	30.5	**< 0.001**	***	-34.90	6.66	1.21
	Apr	20.7	**< 0.001**	***	-37.97	5.91	1.30
	May	8.8	**0.004**	**	-30.58	3.92	1.32
	Jun	1.7	0.191		-6.96	1.61	1.22
	Jul	4.2	**0.043**	*	44.49	-1.66	0.81
	Aug	5.8	**0.018**	*	47.80	-1.85	0.76
	Sep	3.1	0.083		50.08	-2.15	1.23
	Oct	27.0	**< 0.001**	***	-87.59	8.81	1.69
	Nov	24.2	**< 0.001**	***	-22.35	5.03	1.02
	Dec	19.8	**< 0.001**	***	-3.11	3.70	0.83
	Annual	19.3	**< 0.001**	***	-72.18	8.17	1.86

Table 3.1. (Continued)

Variables	Month	$F_{1,83}$	p-value		Est. intercept	Est.slope	SE (slope)
Tmean	Jan	29.0	**< 0.001**	***	-19.49	4.00	0.74
	Feb	33.8	**< 0.001**	***	-31.44	4.58	0.79
	Mar	26.4	**< 0.001**	***	-41.45	4.69	0.91
	Apr	12.3	**0.001**	***	-28.89	3.17	0.90
	May	3.0	0.088		-7.96	1.38	0.80
	Jun	0.0	0.972		16.39	-0.02	0.66
	Jul	2.7	0.105		33.17	-0.73	0.44
	Aug	4.4	**0.038**	*	36.27	-0.86	0.41
	Sep	0.6	0.438		3.54	0.56	0.71
	Oct	20.1	**< 0.001**	***	-56.87	4.31	0.96
	Nov	32.1	**< 0.001**	***	-34.07	4.48	0.79
	Dec	24.6	**< 0.001**	***	-12.64	3.46	0.70
	Annual	9.3	**0.003**	**	-37.18	3.38	1.11
DewTmean	Jan	23.8	**< 0.001**	***	3.95	4.26	0.87
	Feb	22.9	**< 0.001**	***	2.73	4.49	0.94
	Mar	26.7	**< 0.001**	***	-5.97	5.49	1.06
	Apr	27.2	**< 0.001**	***	-14.65	6.02	1.16
	May	24.3	**< 0.001**	***	-26.39	5.99	1.22
	Jun	22.1	**< 0.001**	***	-51.41	7.20	1.53
	Jul	12.1	**0.001**	***	-64.01	7.14	2.06
	Aug	10.4	**0.002**	**	-53.68	6.12	1.90
	Sep	19.1	**< 0.001**	***	-40.29	5.82	1.33
	Oct	22.2	**< 0.001**	***	-23.63	5.37	1.14
	Nov	22.8	**< 0.001**	***	-9.59	5.11	1.07
	Dec	21.3	**< 0.001**	***	5.60	4.11	0.89
	Annual	24.9	**< 0.001**	***	-24.30	6.17	1.24
VPDmax	Jan	12.3	**< 0.001**	***	-4.32	5.80	1.66
	Feb	18.5	**< 0.001**	***	-15.63	6.83	1.59
	Mar	8.2	**0.005**	**	-4.42	3.62	1.26
	Apr	6.8	**0.011**	*	-1.87	2.43	0.93
	May	1.5	0.217		7.71	0.83	0.67
	Jun	0.0	0.920		15.41	0.00	0.45
	Jul	2.7	0.104		22.50	-0.41	0.25
	Aug	3.3	0.071		22.51	-0.42	0.23
	Sep	0.2	0.627		18.54	-0.17	0.35
	Oct	3.8	0.056		2.95	1.47	0.76
	Nov	16.8	**< 0.001**	***	-8.45	6.18	1.51
	Dec	9.7	**0.003**	**	0.71	4.92	1.58
	Annual	0.1	0.775		14.00	0.22	0.77

Table 3.2. Significance of interaction with latitude between SNC disease severity index and climate variables on SNCC study plots in coastal western Oregon and southwestern Washington in 2013-2015. If the interaction effect is significant, that means the association between SNC disease severity index and climate variables differs by latitude.

		Climate effect		Latitude effect		Interaction	
Variables	Month	$F_{1,78}$	p-value	$F_{5,16}$	p-value	$F_{5,78}$	p-value
Precipt	Jan	3.7	**0.059**	4.7	**0.008**	0.7	0.629
	Feb	5.2	**0.026**	4.3	**0.011**	0.8	0.560
	Mar	4.8	**0.031**	3.9	**0.017**	0.5	0.784
	Apr	2.2	0.141	4.4	**0.011**	0.5	0.789
	May	2.6	0.110	4.3	**0.012**	0.5	0.794
	Jun	0.0	0.833	4.5	**0.009**	0.5	0.774
	Jul	9.4	**0.003**	3.1	**0.040**	0.4	0.827
	Aug	1.1	0.291	4.3	**0.011**	0.4	0.838
	Sep	0.7	0.400	4.8	**0.007**	0.7	0.646
	Oct	0.0	1.000	4.8	**0.007**	0.5	0.780
	Nov	0.7	0.396	4.9	**0.006**	0.4	0.844
	Dec	6.9	**0.010**	4.1	**0.014**	0.7	0.611
	Annual	2.3	0.131	4.6	**0.009**	0.6	0.738
Tmin	Jan	1.0	0.314	10.0	**0.000**	3.2	**0.012**
	Feb	2.9	0.090	10.3	**0.000**	3.5	**0.007**
	Mar	0.4	0.516	11.7	**0.000**	4.1	**0.002**
	Apr	0.5	0.474	13.8	**< 0.001**	4.1	**0.002**
	May	1.3	0.249	12.8	**< 0.001**	2.9	**0.018**
	Jun	1.9	0.173	10.0	**0.000**	2.4	**0.047**
	Jul	17.6	**0.000**	2.0	0.141	0.6	0.706
	Aug	14.9	**0.000**	2.6	**0.070**	0.3	0.918
	Sep	21.4	**< 0.001**	1.2	0.349	0.2	0.940
	Oct	10.6	**0.002**	4.5	**0.010**	0.6	0.720
	Nov	5.5	**0.021**	8.3	**0.001**	1.4	0.242
	Dec	3.0	0.089	8.8	**< 0.001**	2.3	0.056
	Annual	5.8	**0.018**	9.8	**< 0.001**	2.7	**0.028**
Tmax	Jan	0.3	0.612	12.9	**< 0.001**	2.2	0.058
	Feb	0.1	0.741	15.7	**< 0.001**	2.7	**0.026**
	Mar	0.1	0.722	16.5	**< 0.001**	2.7	**0.028**
	Apr	2.9	0.091	11.2	**< 0.001**	2.4	**0.047**
	May	2.1	0.155	8.1	**0.001**	4.3	**0.002**
	Jun	0.0	0.915	5.4	**0.004**	1.8	0.122
	Jul	7.4	**0.008**	3.3	**0.031**	1.1	0.344
	Aug	7.8	**0.007**	4.8	**0.007**	0.3	0.865
	Sep	11.1	**0.001**	3.3	**0.031**	3.4	**0.008**
	Oct	5.3	**0.024**	13.6	**< 0.001**	1.7	0.136
	Nov	1.1	**0.294**	13.9	**< 0.001**	2.4	**0.043**
	Dec	0.9	0.334	10.8	**< 0.001**	1.9	0.100
	Annual	2.3	0.135	11.8	**< 0.001**	2.7	**0.027**

Table 3.2. (Continued)

		Climate effect		Latitude effect		Interaction	
Variables	Month	$F_{1,78}$	p-value	$F_{5,16}$	p-value	$F_{5,78}$	p-value
Tmean	Jan	0.1	0.760	14.2	**< 0.001**	1.5	0.216
	Feb	0.3	0.559	16.6	**< 0.001**	1.9	0.097
	Mar	2.9	0.095	13.5	**< 0.001**	2.4	**0.042**
	Apr	5.3	**0.025**	7.3	**0.001**	2.4	**0.048**
	May	0.6	0.432	6.3	**0.002**	4.0	**0.003**
	Jun	1.1	0.297	4.9	**0.007**	1.4	0.244
	Jul	4.0	**0.050**	4.0	**0.016**	1.4	0.241
	Aug	4.0	**0.050**	5.2	**0.005**	0.6	0.713
	Sep	0.0	0.864	5.5	**0.004**	5.7	**< 0.001**
	Oct	0.3	0.613	10.6	**< 0.001**	1.8	0.133
	Nov	0.0	0.980	16.3	**< 0.001**	2.1	0.072
	Dec	0.3	0.562	12.5	**< 0.001**	1.6	0.180
	Annual	0.0	0.842	8.2	**0.001**	3.7	**0.005**
DewTmean	Jan	4.6	**0.036**	12.2	**< 0.001**	2.9	0.019
	Feb	0.9	0.348	14.3	**< 0.001**	4.0	**0.003**
	Mar	4.3	**0.041**	15.1	**< 0.001**	4.0	**0.003**
	Apr	9.5	**0.003**	14.7	**< 0.001**	5.0	**0.001**
	May	10.0	**0.002**	13.7	**< 0.001**	4.5	**0.001**
	Jun	9.2	**0.003**	12.4	**< 0.001**	4.2	**0.002**
	Jul	8.4	**0.005**	7.9	**0.001**	3.5	**0.007**
	Aug	16.8	**< 0.001**	6.8	**0.001**	5.5	**< 0.001**
	Sep	19.8	**< 0.001**	9.8	**0.000**	8.8	**< 0.001**
	Oct	10.3	**0.002**	13.0	**< 0.001**	6.0	**< 0.001**
	Nov	3.0	0.088	15.0	**< 0.001**	4.7	**0.001**
	Dec	2.3	0.131	11.7	**< 0.001**	2.7	**0.025**
	Annual	7.4	**0.008**	13.9	**< 0.001**	4.8	**0.001**
VPDmax	Jan	5.2	**0.026**	9.4	**< 0.001**	0.9	0.508
	Feb	0.6	0.446	11.0	**< 0.001**	2.1	**0.076**
	Mar	5.7	**0.020**	6.5	**0.002**	3.4	**0.008**
	Apr	5.8	**0.019**	4.9	**0.006**	1.9	0.111
	May	1.9	0.175	5.0	**0.006**	3.8	**0.004**
	Jun	0.1	0.711	5.1	**0.006**	1.3	0.253
	Jul	3.2	0.077	4.3	**0.011**	1.2	0.303
	Aug	3.2	0.079	5.3	**0.005**	0.5	0.771
	Sep	0.2	0.663	4.5	**0.009**	5.2	**< 0.001**
	Oct	0.3	0.588	5.5	**0.004**	1.2	0.298
	Nov	0.9	0.348	11.3	**< 0.001**	1.7	0.140
	Dec	4.0	**0.049**	8.3	**0.001**	0.3	0.914
	Annual	0.0	0.908	4.3	**0.012**	2.5	**0.038**

Table 3.3. Significance of interaction with longitude between SNC disease severity index and climate variables on SNCC study plots in coastal western Oregon and southwestern Washington in 2013-2015. If the interaction effect is significant, that means the association between SNC disease severity index and climate variables differs by longitude.

		Climate effect		Longitude effect		Interaction	
Variables	Month	$F_{1,80}$	p-value	$F_{3,18}$	p-value	$F_{3,80}$	p-value
Precipt	Jan	5.9	**0.017**	0.7	0.559	0.3	0.835
	Feb	6.8	**0.011**	0.7	0.547	0.4	0.723
	Mar	6.1	**0.016**	0.9	0.447	0.4	0.780
	Apr	3.9	**0.051**	0.7	0.573	0.8	0.515
	May	4.0	**0.049**	0.9	0.440	0.5	0.688
	Jun	1.3	0.259	0.7	0.579	0.3	0.856
	Jul	7.3	**0.008**	1.0	0.403	0.6	0.612
	Aug	0.0	0.872	0.6	0.642	0.5	0.710
	Sep	0.0	0.866	0.6	0.632	0.3	0.816
	Oct	0.9	0.336	0.5	0.663	0.4	0.782
	Nov	2.7	0.103	0.6	0.642	0.2	0.921
	Dec	7.9	**0.006**	0.8	0.503	0.5	0.715
	Annual	4.4	**0.038**	0.7	0.576	0.3	0.852
Tmin	Jan	0.4	0.522	2.3	0.114	0.6	0.606
	Feb	1.3	0.253	2.8	0.067	0.8	0.490
	Mar	0.3	0.599	0.5	0.673	0.5	0.654
	Apr	4.2	**0.045**	0.2	0.931	0.3	0.824
	May	5.1	**0.027**	0.2	0.929	0.6	0.604
	Jun	4.4	**0.040**	0.3	0.857	0.4	0.732
	Jul	20.2	**< 0.001**	1.4	0.270	1.8	0.146
	Aug	17.2	**< 0.001**	1.9	0.163	3.4	**0.022**
	Sep	30.2	**< 0.001**	4.1	**0.022**	1.7	0.181
	Oct	11.2	**0.001**	6.6	**0.003**	1.8	0.164
	Nov	3.4	**0.071**	3.8	**0.028**	0.8	0.506
	Dec	1.9	0.173	3.3	**0.044**	0.8	0.499
	Annual	3.0	0.085	3.6	**0.033**	1.3	0.292
Tmax	Jan	3.3	0.074	0.2	0.912	0.4	0.767
	Feb	5.9	**0.017**	0.2	0.910	0.3	0.800
	Mar	8.4	**0.005**	0.1	0.934	0.1	0.977
	Apr	10.7	**0.002**	0.2	0.893	0.4	0.785
	May	7.1	**0.009**	0.6	0.653	0.3	0.809
	Jun	2.1	0.151	0.8	0.512	0.7	0.556
	Jul	4.9	**0.031**	0.4	0.787	1.3	0.295
	Aug	2.3	0.131	0.3	0.833	1.6	0.200
	Sep	8.7	**0.004**	0.4	0.728	0.9	0.423
	Oct	1.4	0.234	0.3	0.819	1.7	0.169
	Nov	1.6	0.215	0.2	0.871	0.2	0.900
	Dec	0.1	0.759	0.6	0.636	0.3	0.811
	Annual	3.7	0.059	0.3	0.843	0.8	0.521

Table 3.3. (Continued)

		Climate effect		Longitude effect		Interaction	
Variables	Month	$F_{1,80}$	p-value	$F_{3,18}$	p-value	$F_{3,80}$	p-value
Tmean	Jan	6.6	**0.012**	0.2	0.895	0.6	0.635
	Feb	10.9	**0.001**	0.2	0.908	0.3	0.807
	Mar	12.5	**< 0.001**	0.2	0.882	0.3	0.829
	Apr	10.1	**0.002**	0.2	0.642	0.2	0.900
	May	3.8	**0.054**	0.9	0.466	0.2	0.886
	Jun	0.5	0.485	0.8	0.521	0.9	0.441
	Jul	0.6	0.442	0.4	0.742	1.5	0.226
	Aug	0.4	0.519	0.4	0.740	1.7	0.172
	Sep	2.5	0.121	1.0	0.422	0.8	0.518
	Oct	12.3	**< 0.001**	0.3	0.813	1.6	0.204
	Nov	8.7	**0.004**	0.2	0.902	0.4	0.761
	Dec	3.4	0.068	0.2	0.906	0.5	0.675
	Annual	5.9	**0.017**	0.5	0.659	0.6	0.632
DewTmean	Jan	8.6	**0.004**	0.3	0.854	0.2	0.896
	Feb	4.8	**0.032**	0.1	0.935	0.1	0.954
	Mar	9.2	**0.003**	0.2	0.929	0.2	0.910
	Apr	12.1	**0.001**	0.1	0.947	0.2	0.900
	May	11.7	**0.001**	0.1	0.965	0.2	0.905
	Jun	12.3	**0.001**	0.1	0.967	0.8	0.499
	Jul	10.5	**0.002**	0.3	0.839	2.0	0.127
	Aug	9.5	**0.003**	0.3	0.830	1.0	0.378
	Sep	12.0	**0.001**	0.1	0.969	1.1	0.356
	Oct	9.8	**0.002**	0.1	0.958	0.2	0.893
	Nov	7.1	**0.009**	0.1	0.939	0.1	0.976
	Dec	5.3	**0.023**	0.2	0.905	0.2	0.926
	Annual	10.3	**0.002**	0.1	0.937	0.2	0.908
VPDmax	Jan	0.4	0.548	0.9	0.457	0.1	0.983
	Feb	7.6	**0.007**	0.3	0.857	0.7	0.574
	Mar	7.8	**0.007**	0.9	0.468	1.0	0.411
	Apr	7.9	**0.006**	1.1	0.391	0.2	0.900
	May	3.6	0.062	1.4	0.290	0.1	0.958
	Jun	1.3	0.261	1.0	0.432	1.2	0.330
	Jul	0.4	0.513	0.5	0.720	1.3	0.291
	Aug	0.2	0.669	0.5	0.679	1.9	0.143
	Sep	0.7	0.397	0.9	0.451	1.0	0.392
	Oct	2.5	0.118	0.6	0.639	1.7	0.167
	Nov	3.2	0.078	0.3	0.862	0.9	0.467
	Dec	0.5	0.500	1.0	0.411	0.2	0.924
	Annual	1.3	0.266	0.9	0.459	1.1	0.374

Table 3.4. Key findings from previous studies that have examined relationships between Swiss needle cast severity and weather variables.

Literature	Climate variable	Range of climate variables	Association
Our study	Tmean and Tmax	Oct-Apr	positive
	Precipitation	Dec-Mar	negative
	Dew point temperature	all months	positive
	Tmin	all months except Oct	various
	VPDmax	Nov-Apr	positive
Black et al. 2010	Spring temperature Summer temperature	Mar-Aug	positive
Lee et al. 2013	Winter temperature	Jan-Mar or Dec-Feb	positive
	Summer temperature	Jun-Jul	negative
	Summer precipitation	Jun-Jul	positive
	Summer dew point deficit	Jun-Jul	negative
Lee et al. 2016	High temperature with wetter winter and drier summer		Positive/negative
	Summer dew point deficit	Jun-Sep	positive
Lee et al. 2017	Winter temperature	various on site, Dec-Feb, Jan-Mar, or Feb-Apr	positive
	Summer temperature	Jun-Jul	negative
	Summer precipitation	Jun-Jul	positive
Manter et al. 2005	Winter temperature	Dec-Feb	positive
	Leaf wetness	May-Jul	positive
Rosso and Hansen 2003	Summer humidity	July	positive
	Summer Tmean	July	negative
Stone et al. 2007	Aug Tmin (Winter in New Zealand)	Aug	positive
	June Tmean (Winter in New Zealand)	Jun	positive
Stone et al. 2008	Winter temperature	Dec-Feb	positive
Watt et al. 2010	June air temperature (Winter in New Zealand)	Jun	positive
	Nov precipitation (Spring in New Zealand)	Nov	positive
Wilhelmi et al. 2017	May-Sep precipitation	May-Sep	positive
	Winter temperature	Dec-Feb	positive
Zhao et al. 2012	Spring precipitation	Mar-May	positive
	Winter Tmin	Dec-Feb	positive

CHAPTER 4 – SERVERITY OF SWISS NEEDLE CAST IN YOUNG VERSUS OLD DOUGLAS-FIR FOREST IN WESTERN OREGON, USA

Abstract

Swiss needle cast (SNC) is an important foliage disease of Douglas-fir (*Pseudotsuga menziesii*) forests in the coastal region of the Pacific Northwest. While there is considerable evidence of SNC disease in coastal Douglas-fir plantations, the severity of SNC in mature and old-growth forests is poorly understood. We compared the SNC severity and incidence, needle retention, and foliar nitrogen in tree crowns of mature and old-growth forests and nearby young forests at three locations in the Oregon Coast Range and four locations in the western Cascade Mountains of Oregon. Disease severity, as assessed on 2-year old needles, was greater in younger forests than older forests at all sites. Retention of 1-4 year-old needle cohorts did not differ between young and old trees, but older trees had much larger complements of >4 year-old needles. Incidence of disease was highest for 2-year-old needles in young trees and 3-5 year-old needles in older trees. Total foliar nitrogen concentration did not differ in needles of young and old trees, but at some locations total N differed between canopy positions. Leaf wetness differences were not consistent between young and old tree crowns and did not explain disease severity differences. However, at one study site in the core epidemic area, the younger stand had longer periods of wetness in the upper crowns than a nearby old stand. Leaf wetness and foliar N were hypothesized to play a role in SNC disease severity, but apparently these are not controlling factors. In younger stands, the fungus appeared to block stomates earlier than in older stands and stomatal occlusion was always greater on younger than older trees for 2 year old needles. We speculate that multiple factors may have caused the observed differences, including differences in thermal

properties of older and younger stands, needle anatomy, chemical differences, or genetics of old tree and young trees. It is also possible that older trees are less impacted by SNC because they have experienced exposure to the disease over a longer period of time, and this influences host-fungus interactions. Also, four of the sites that we examined were outside the current epidemic area and were not considered diseased, so this may have influenced needle retention. The relationships observed in our study need testing with larger samples to determine if our results are generally applicable to Douglas-fir in western Oregon.

Introduction

Swiss needle cast, caused by *Nothophaeocryptopus gaeumannii* (T. Rohde) Videira et al. (Ascomycete: Mycosphaerellaceae), is an important foliage disease of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantations in the coastal region of the Pacific Northwest (Hansen et al. 2000, Shaw et al. 2011). Cooperative aerial detection surveys across coastal Oregon and Washington have shown the disease to be intensifying, with 1996 aerial survey in Oregon detecting 53,050 ha, increasing to 238,705 ha in 2015 (Ritóková et al. 2016). Rather than directly attacking host cells, *N. gaeumannii* is an endophyte which causes disease by carbon starvation, the reproductive structures (pseudothecia) plug the stomates and prevent carbon uptake and transpiration (Manter et al. 2000). Disease impact in forest plantations is associated with loss of foliage. Foliage retention of less than 3 years results in reduced tree volume growth , foliage retention of 2 years is associated with a >25% volume loss (Maguire et al. 2002, 2011). Mortality is rare, but stands with ~1 year or less of foliage retention are associated with the most severe disease expression (Maguire et al. 2011).

Needle retention is a key factor in assessing disease impacts, but older trees typically retain foliage much longer than young trees. Needle retention is also known to vary with site productivity and elevations. The lowest productivity sites and highest elevations have the greatest needle retention. The overall effect of SNC is to lower needle retention across this gradient (Shaw et al. 2014), but the influence of SNC on needle retention in older stands is not known.

Disease severity, assessed on 2-year-old needles, varies with canopy position in younger forests, with severity greatest in the upper crown (Hansen et al. 2000, Manter et al. 2003, Shaw et al. 2014). This is unusual for a foliage disease because foliage disease severity is typically greatest in the most humid portion of the crown, which for conifers is typically the lower and inner crown. Therefore, the assumption has been that leaf wetness is not a controlling factor within the epidemic area (~ within 50 km of the coast). However, leaf wetness and humidity are necessary for spore dispersal, germination on the leaf, and growth of the hyphae into stomates (Manter et al. 2005).

Epidemiology of SNC has focused on winter temperature and leaf wetness during spore dispersal from May through August (Manter et al. 2005). Subsequent models using needle retention found that the possibility of needle survival was positively related to minimum winter temperature (Dec-Feb) and spring (Mar-May) precipitation (Zhao et al. 2012). Dendrochronological analysis has shown that mature forests in the coastal mountains are susceptible to growth reduction by SNC in the past, specifically associated to warmer spring and summer temperature (Black et al. 2010). Forests further from the coast in the Cascade Mountains also have been reported with SNC, particularly the low elevation foolhills (Ritóková et al. 2016). Data from 59 stands across the northwestern Oregon Cascade Mountains has shown that disease severity strongly decreased with increasing elevation (Ritóková et al. 2016). Lee et al. (2013) demonstrated that winter and summer temperatures and summer precipitation were strongly correlated with SNC impacts on basal area growth in mature and old-growth forests in the Cascade Mountains.

In addition, the relative importance of these climate variables varied by elevation and distance from the coast (Lee et al. 2016, 2017).

Anecdotal observations suggest that SNC is more severe in young trees than in mature trees, however, this has not been measured quantitatively. SNC has been well studied in young-growth stands but there is still uncertainty regarding what controls disease severity beyond weather conditions. Data on SNC severity in mature and old-growth forests are rare and there is little understanding of SNC disease ecology in older forests. We hypothesized that the vertical and horizontal complexity of older stands would lead to differentiation of microclimate within the vertical profile of older tree crowns and that would lead to less uniform infection of the crown by *N. gaeumannii*. We predicted that this could result in less severe SNC disease in old forest.

Two factors thought to be influential in fungal disease ecology in conifer forests are leaf wetness during spore dispersal (Capitano 1999) and nitrogen content of the leaf (El-Hajj et al. 2004). These factors are expected different in crowns of young versus older trees because of differences in tree morphology, needle age composition and microclimate within the tree. Given that, we hypothesized that wetter needles have higher disease severity and higher nitrogen content would be associated with higher disease.

In this study, we investigated SNC disease patterns in tree crowns of mature and old-growth forests and nearby young forests at three locations in the Oregon Coast Range and four locations in the western Cascade Mountains. We compared needle samples from old and young trees at each site to determine if SNC disease severity differed between age classes. We also compared infection incidence in different needle age classes, needle retention, and foliage nitrogen patterns and leaf wetness (May – August) to determine if these variables differed between tree age classes.

Methods

Study sites

Foliage samples were collected in 2016 and 2017 at seven sites in western Oregon, including five sites in long-term ecological monitoring plots established by the Environmental Protection Agency (hereafter EPA; Lee et al. 2016) and 2 sites in the Siuslaw National Forest (Fig. 4.1). Four sites were located on the west slope of the Cascade Range (Moose Mountain, Fall Creek, Toad Creek, and Soapgrass Mountain), and three sites were in the Coast Range (Cascade Head, Woods Creek, and Klickitat Mountain). Forests at each site included a mixture of old stands of Douglas-fir that were 114-470 years old and young stands of Douglas-fir that were 20-30 years old. The old forests were unmanaged stands that regenerated after fires, whereas the young stands were plantations, growing on areas that had been clear-cut and replanted. Elevation ranged from 140m at the lowest plot in the Coast Range to 1200m at the highest plot in the Cascade Range. Precipitation varied from 1300-2700 mm (Table 4.1). Associated tree species included western hemlock and western red cedar, as well as Pacific silver fir and noble fir at higher elevations in the Cascades (Table 4.2).

Field Sampling

Trees were sampled by climbing on ropes and collecting branch samples at three levels in the tree crown (lower, middle, upper). In the two stands that did not have EPA weather stations, we selected sample trees that had well-developed crowns and were easily accessible (2 sites × 3 trees = 6 old trees and 6 young trees) without placing leaf wetness sensors in canopies. In each old forest stand monitored by the EPA, we climbed three trees that had been previously rigged for climbing (5 sites × 3 old trees). In each young stand monitored by the EPA, we chose 3 trees for sampling based primarily on whether they had a weather station installed nearby (5 sites × 3 young trees). Weather stations had previously been installed in trees on 5 sites managed by the EPA, including Cascade Head in the Coast Range, and Moose Mountain, Falls Creek, Soapgrass Mountain and Toad Creek in the western Cascades. In each mature stand, the EPA selected one tree in which they installed one weather station at the base of the tree and another weather station in the top of the tree. In each young stand, the EPA installed a single weather station that was placed 2 m above ground. In addition to the weather stations maintained by the EPA, we installed leaf wetness sensors in one old and one young tree at each site, with sensors placed along the vertical gradient of each tree at the upper, middle and lower canopy positions where we sampled branches for SNC.

We collected 1-3 branches from three canopy positions (lower, middle, and upper crown) in each tree. Samples were collected on the south side of the tree in late May through early June, after bud-breaking and before new needles were elongated. At least one branch >1 meter in length was selected to ensure sufficient needle material for measurements and foliage nutrient analysis. Several shorter branches were chosen if there were no branches greater than 1 meter in length. Limbs were transported to the lab and stored in a 5˚C cold room. Needle retention was determined by estimating the number of years (annual cohorts) of foliage present on a stem that was >4-year-old (Maguire et al. 2011).

Lab Analysis

For each canopy height level, 50 individual needles were randomly selected from each age class. Needles were taped on an index card and stored at -20 ˚C. All age needles were then examined under a microscope for evidence of occluded stomates and presence or absence of pseudothecia, which is called incidence in this study. The first 10 two-year-old needles with pseudothecia present were then examined to determine the % of stomates occluded in three regions (base, mid, and tip) of the needle. In each region, we picked a starting point from the needle base based on a random number's table and examined 100 stomates from the starting point to determine the number that were occluded by pseudothecia. Pseudothecial occlusion in the three regions was then averaged for each needle and then averaged for 10 needles per canopy level per tree. The SNC disease severity index was calculated as the ratio of occluded stomates × the percentage of needles with pseudothecia for the 50 needles.

Foliar nitrogen was determined on dried and ground foliar material using dry combustion in a FlashEA 1112 NC Analyzer (Thermo Fisher Scientific Inc. USA). Only one-year-old needles were collected for foliage nitrogen measurements. After transporting limbs to the lab, we randomly selected ~ 200 needles from each canopy position, dried them for 48 hours in a drying oven at 40 ˚C, and ground them with a ball grinder and stored in clean vials. We then placed 3-5mg of the powder into a tin capsule and used FlashEA1112 to measure total nitrogen concentration (%).

Leaf Wetness Data Collection

We estimated leaf wetness duration during May-August as the ratio of total wet hours in each month / total hours in each month. Only May-August leaf wetness data were examined because that is the primary period of spore dispersal and leaf colonization (Michaels and Chastagner 1984). Leaf wetness data were collected every 5 minutes with a PHYTOS31 sensor (Decagon Devices, Inc. USA) and recorded as hourly data in mV with a CR1000 datalogger (Campbell Scientific Inc. USA). Base on the excitation voltage for our datalogger (2.5V), we assumed that leaf surfaces were wet when resistance values > 280mV (The manual of leaf wetness sensor is available at http://library.metergroup.com/Manuals/10386_Leaf%20Wetness%20Sensor_Web.pdf, last checked June 5, 2018). In addition, as a quality control of the sensors, we also compared the leaf wetness data with other moisture-related data that were collected on site with rainfall collectors and humidity sensors at Cascade Head, and 280mV was the mean value from all sensors when no moisture was present.

Data Analysis

We used multivariate analysis of variance (MANOVA) to test for the main effects of canopy position (upper, middle, and lower), tree age (mature or young), sites, and years (2016 and 2017), and their interactions with the SNC severity index, needle retention, and total foliar nitrogen. We used alpha = 0.05 as the cutoff for statistical significance. Canopy position and year were treated as within-subject factors in the MANOVA whereas tree age and sites were between-subject factors. The SNC severity index data from Soapgrass Mountain and Toad Creek were excluded from the analysis because almost all SNC values from those sites were zeros (Fig. 4.3). In the preliminary results (Table 4.3), there were interactions involving sites, so we also ran the MANOVA on individual sites, to test for differences in SNC severity index between canopy position, year, and tree age (Table 4.4). The post MANOVA in the latter analysis we conducted a mean separation test to compare paired main factor levels if interactions were not present. MANOVA tests were performed using R (v. 3.4.3, R Core Team 2017) and package car (Fox and Weisberg 2011), dplyr (Wickham et al. 2017), emmeans (Lenth 2018), ggplot2 (Wickham 2009), and nlme (Pinheirro, et al. 2017).

Results

SNC incidence patterns

The percentage of needles with pseudothecia (i.e., SNC incidence) varied by site, tree age, and canopy position (Fig. 4.2). SNC incidence was lowest in mature trees at high-elevation sites in the Cascades (Soapgrass Mountain and Toad Creek), and highest in young and mature trees at Cascade Head in the Coast Range. At all study sites SNC incidence was greater in young stands than in the paired old forest stands. At all sites excluding Soapgrass Mountain and Toad Creek, nearly 100% of the two-year-old and older needles in young trees had *N. gaeumannii* present, whereas the peak of incidence in mature trees was observed in 3-5-year-old needles, followed by a decline with increasing needle age. Also, there was more variation in SNC incidence among old trees than in the paired young trees, which means the young trees were more evenly infected by *N. gaeumannii* than were old trees.

In young trees, *N. gaeumannii* was present on almost all needles older than 2 years in all three canopy positions. In mature trees, the percentage of needles with *N. gaeumannii* present was higher in the middle and lower canopy than in the upper canopy, At Soapgrass Mountain and Toad Creek, there were some lower canopy needles with at least one stomate occluded by *N. gaeumannii* in Soapgrass Mountain young trees, but most needles from these two sites did not have any pseudothecia (Fig. 4.2). There was a unique SNC incidence pattern that only occurred in mature trees in most sites: 3-5 year-old needles had the highest SNC incidence (Fig. 4.2). These were the needle cohorts that emerged in 2011-2013.

SNC severity index

The SNC severity index on 2-year-old needles varied by site, tree age, canopy position, and year (Fig. 4.3). The SNC severity index was nearly 0% for young trees at Soapgrass Mountain and Toad Creek and for old trees at all sites except Cascade Head and Klickitat Mountain. The MANOVA excluded the data for Soapgrass Mountain and Toad Creek due to lack of variation. When the Soapgrass Mountain and Toad Creek sites were removed from the analysis, differences in SNC severity between sites, tree ages, canopy positions, and years were all significant ($p<0.05$, Table 4.3). In particular, SNC severity was greater in young trees than in mature trees ($p<0.001$). Several interactions involving tree age were noted, but were of minor importance because their F-values were an order of magnitude less than that for tree age (Snedecor and Cochran, 1967). Several interactions involving site, year, and/or canopy position were also statistically significant ($p<0.05$, Table 4.3). Based on the mean separation test on site with Bonferroni adjustment, the SNC severity index was different in Cascade Head than in other 4 sites (Fig. 4.4). Consequently, MANOVA was performed on the SNC severity index by site to test for the main effects of tree age, canopy, and year, and their interactions (Table 4.4).

Differences in SNC severity among canopy positions were statistically significant at Cascade Head ($p=0.017$) and Woods Creek ($p=0.029$) (Table 4.4). In young and old trees at Cascade Head, the SNC severity index was significantly greater in the upper canopy than in the lower and middle canopies in 2016 and 2017 (Fig. 4.3). At all sites excluding Cascade Head, SNC severity index values for young trees were greater than for old trees. For young trees at Woods Creek, the SNC severity index was significantly lower in the upper canopy in 2016 than in the lower and middle canopy, but was uniformly low in all three canopy layers in 2017. For young trees at Woods Creek, Klickitat Mountain, and Moose Mountain, the mean SNC severity index was greater in 2016 than in 2017 (Fig. 4.3).

Foliage retention

Mean foliage retention differed among sites ($p<0.001$) and canopy positions ($p<0.001$), and included an interaction between sites and canopy position ($p=0.04$) (Fig. 4.5, Table 4.3). Mean foliage retention did not differ among coast and inland sites (Fig. 4.6). Based on the mean separation test on site with Bonferroni adjustment, mean foliage retention was different in Cascade Head and Klickitat Mountain than in other sites (Fig. 4.6), and was lower in the upper canopy than in the middle and lower canopy layers (Fig. 4.7). After removing Soapgrass Mountain and Toad Creek from the analysis, the interaction between sites and canopy was not significant, indicating that foliage retention in those two sites differed from the other areas examined (Table 4.3).

Foliage retention was significantly less in young and mature trees at Cascade Head and Klickitat Mountain than at the five inland sites (Fig. 4.5). Also, foliage retention was lower in the upper canopy than in the lower and middle canopy layers at all sites except for Soapgrass Mountain. The three-factor interaction involving tree age, site, and year was statistically significant based on the MANOVA ($p=0.036$). However, in the analysis of individual sites there was evidence that mean foliage retention differed in young and old trees in Woods Creek and Toad Creek (Table 4.4).

Foliage total nitrogen

Mean total nitrogen concentration differed among years, canopy positions, and sites (Fig. 4.8-4.11, Table 4.3). In addition there was a significant site × year interaction that we did not consider important because its F-value was an order of magnitude less than the F-value for year (Snedecor and Cochran, 1967). Mean total nitrogen concentration was significantly greater in 2016 than in 2017 (Table 4.11), and was greatest in the upper canopy and lowest in the lower canopy (Fig. 4.8). Based on the mean separation test on site with Bonferroni adjustment, mean foliage total nitrogen concentration was different in Cascade Head and Klickitat Mountain than in most other sites (Fig. 4.9), and was higher in the upper canopy than in the middle and lower canopy (Fig. 4.10).

Leaf wetness data

Leaf surfaces were often wet in all canopy positions in all sites during May and June and mostly dry in July and August at Falls Creek, Moose Mountain, and Soapgrass Mountain (Fig. 4.12). There were no obvious patterns in leaf wetness among canopy positions. July 2017 was drier than 2016, but May, June, and August did not show strong differences in leaf wetness between years. Leaf wetness did not differ between sites or tree age classes during May-August. However, young trees at Cascade Head had higher leaf wetness than most other sites in nearly all months. Unfortunately, we did not have leaf wetness data from the other 2 coastal sites so the sample was insufficient for comparisons between coastal and inland sites.

Discussion

Differences between mature and young trees

We found that SNC severity was lower in old trees than in young trees except for the two high elevation sites. These patterns were not well explained by our data on leaf wetness, which indicated that leaf wetness did not differ greatly between tree age categories in May-August. We suspect, therefore, that leaf wetness may not be a controlling factor within the SNC epidemic area. However, at Cascade Head young trees had the highest SNC disease severity, the lowest needle retention, and higher leaf wetness in July and August compared to older trees. This led us to suspect that weather conditions within the epidemic area of SNC near the coast were distinct from the Cascade Mountains.

Foliar nitrogen is thought to be associated with SNC disease severity. El-Hajj et al. (2004) hypothesized that *N. gaeumannii* might acquire nitrogen and carbon from apoplastic spaces within Douglas-fir needles. Perakis et al. (2006) also showed a correlation between increased soil N and disease occurrence, following a pattern of increasing disease east to west in the Oregon Coast Range. Perakis et al. (2006) suggested that higher N was associated with increased foliage disease. We hypothesized that tree age-related differences in foliar nitrogen might influence the severity or occurrence SNC. This hypothesis was not supported by our data, which indicated no difference in foliar nitrogen in the paired samples of old and young trees at individual sites.

Hansen et al. (2000) found that SNC disease severity was highest in the upper canopy of young trees in plantations. We found similar trends at Cascade Head, Klickitat Mountain, and Falls Creek, but disease severity was so low at other sites that we found no patterns relative to disease severity at different levels in the canopy of young trees. In older trees we found some evidence that SNC severity was most pronounced in the middle and upper crown layers, but the disease severity was so rare in old trees in most areas that we were unable to detect consistent patterns. In our study, *N. gaeumannii* was also rare at two high elevation sites. Manter et al. (2005) found that warm winter temperatures were positively associated with SNC, while Ritóková et al. (2016) found a clear relationship with decreasing SNC severity and elevation.

In conifers, older trees have needles that are morphologically different than needles of young trees (Apple et al. 2002, Day et al. 2001, England and Attiwill 2006).These differences may make old Douglas-fir trees more resistant to *N. gaeumannii* infection. Another possibility is that differences in SNC expression between mature and young trees may be influenced by age-related differences in defensive chemicals (Erwin et al. 2001). Secondary chemical compounds are important in plant defense against pathogens (Espinosa-Garcia and Langenheim 1991, Cook and Hain 1986). Another possible explanation for age-related differences in susceptibility to SNC is that old trees that have survived on site may be more resistant to SNC than young trees. Wilhelmi et al. (2017) found that Douglas-fir seedlings from dry sites were more susceptible to SNC when transferred to wet sites. None of the above hypotheses have been tested and they all need further investigation.

Specific patterns on mature trees

The incidence of *N. gaeumannii* on mature trees peaked on 3-5 year-old needles at most sites, and sometimes the peaks shifted from 2016 to 2017. Because *N. gaeumannii* only infects the current needles following bud-break, those infected cohorts could be traced back to 2011-2013, which were years with warmer winters and humid summers (PRISM website http://prism.oregonstate.edu/recent/). The presence of fungus in 3-5 year-old needles in older trees may also result from more time for pseudothecia to form on mature trees. However, it only takes two years for pseudothecia to mature on young trees. More study is needed to understand whether there is a difference in the mechanism of fungal development between mature and young trees.

Most researchers have used 2 year-old needles to investigate the severity of SNC because 2-year-old needles are a good indicator of disease symptoms in young trees (Manter et al. 2005). However, based our study, pseudothecia appeared to be more common on 3-5 year old needles in mature and older trees. This needs further investigation to determine the best approach for assessing disease severity in old trees, especially for inland forests.

Conclusions

Nothophaeocryptopus gaeumannii is a common and ubiquitous endophyte specific to Douglas-fir that occurs everywhere Douglas-fir grows. It causes disease when pseudothecia plug stomates, leading to total needle retention of less than 3 years in young forests, as well as chlorosis and growth reduction. In our study, no matter whether disease was being expressed or not, the severity of disease based on two-year-old needles was always lower in older trees than in young trees in nearby plantations. Our results were less consistent in terms of relationships between fungus infection, foliar nitrogen concentrations, and leaf wetness. The scope of inference in our study is only for 7 locations in western Oregon. Although our results were relatively consistent among sites, more research is needed to better document patterns across all of western Oregon.

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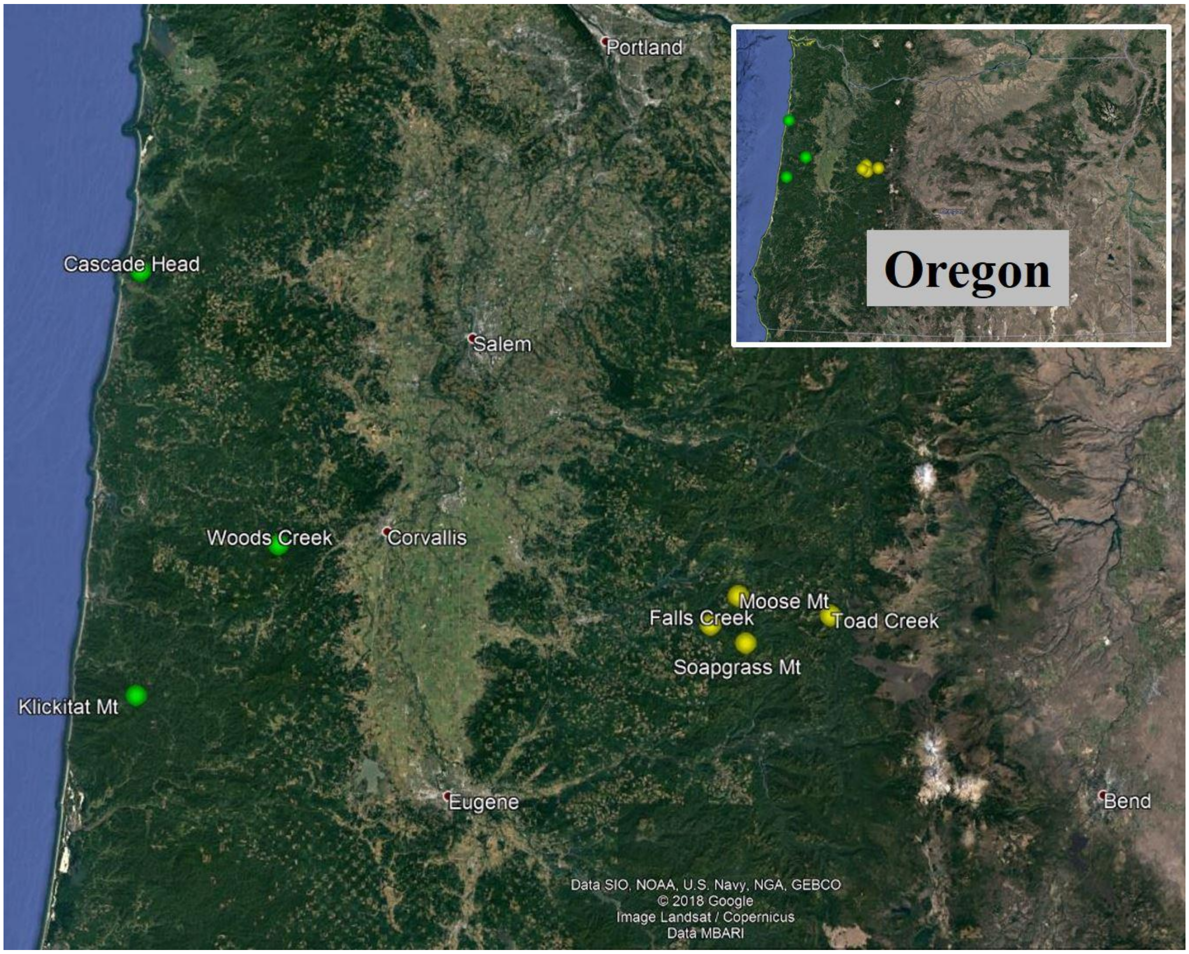
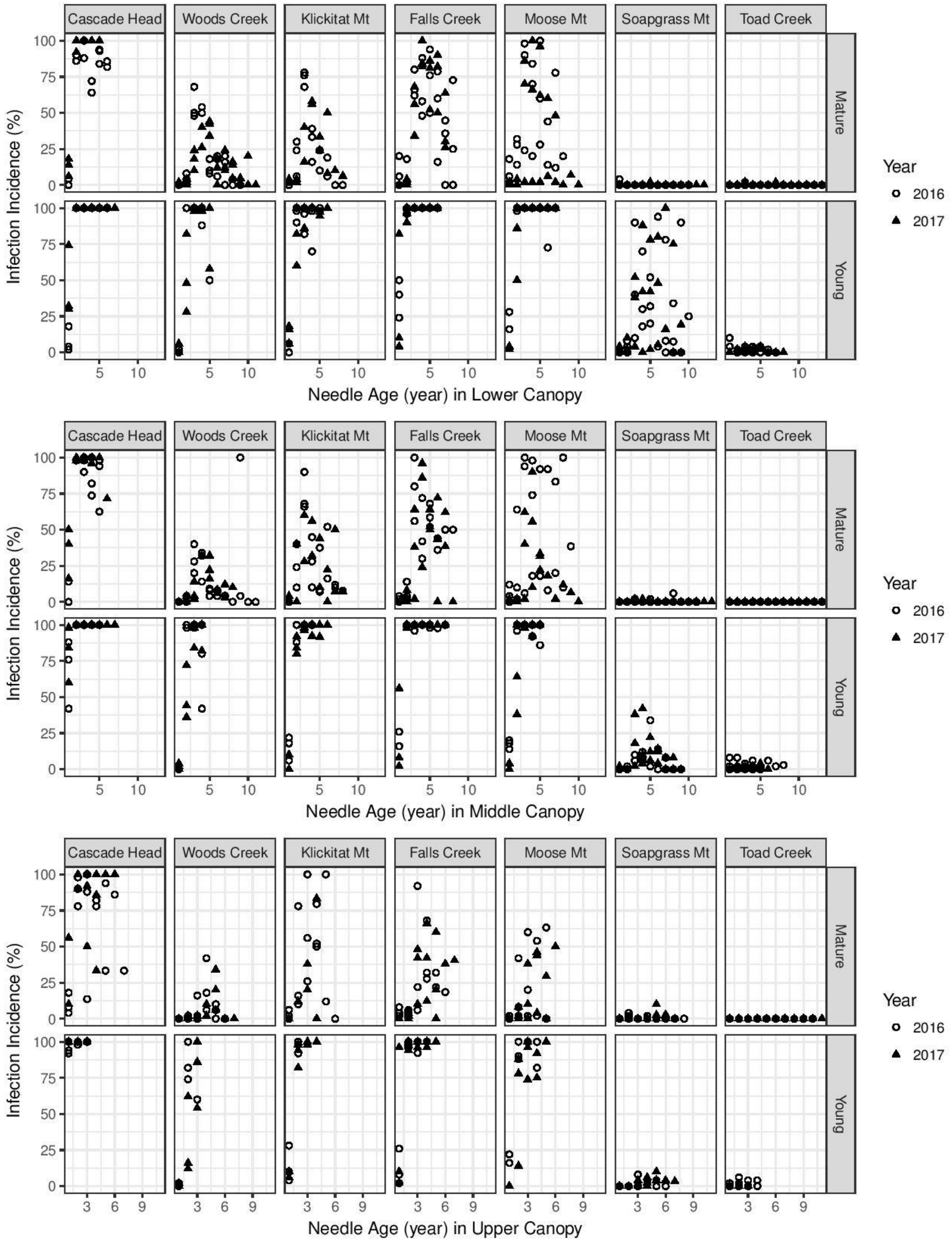


Figure 4.1. Study area with the Oregon Coast Range adjacent to the coast, and the Cascade Mountains to the east. The map was captured from Google Earth in 2018.



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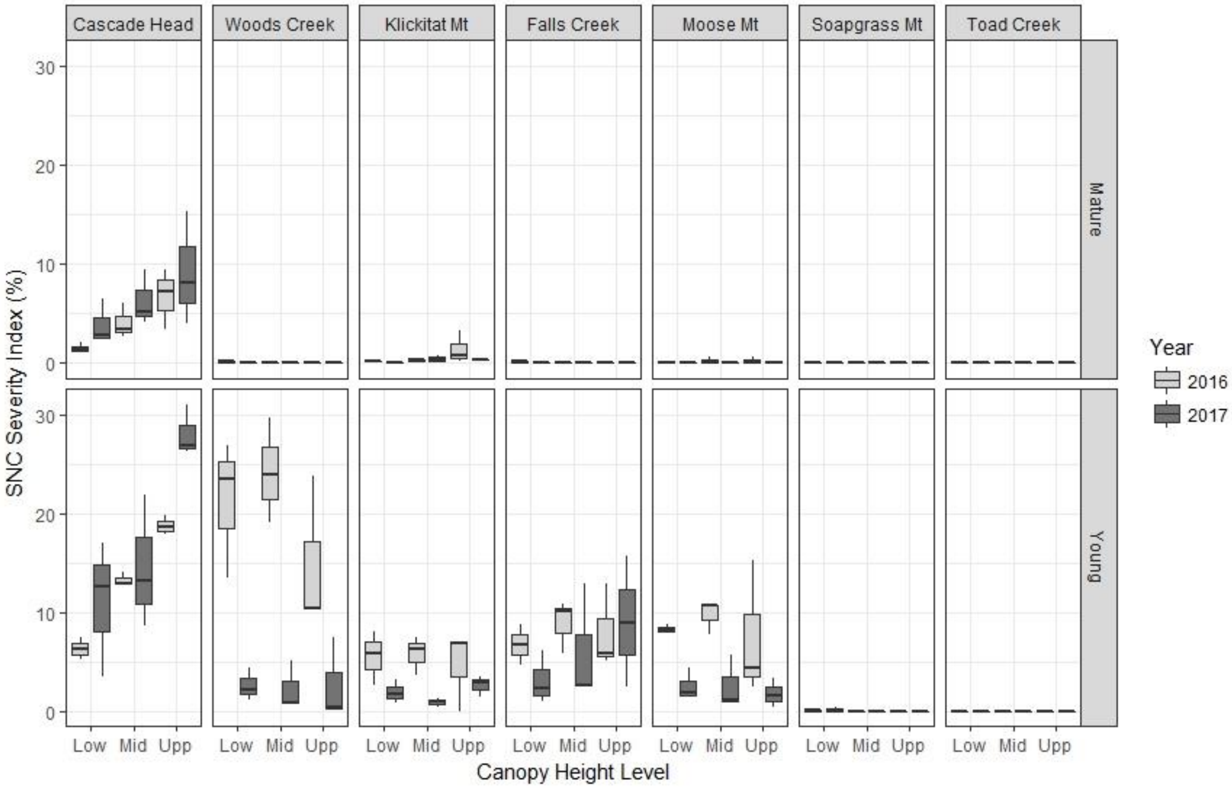

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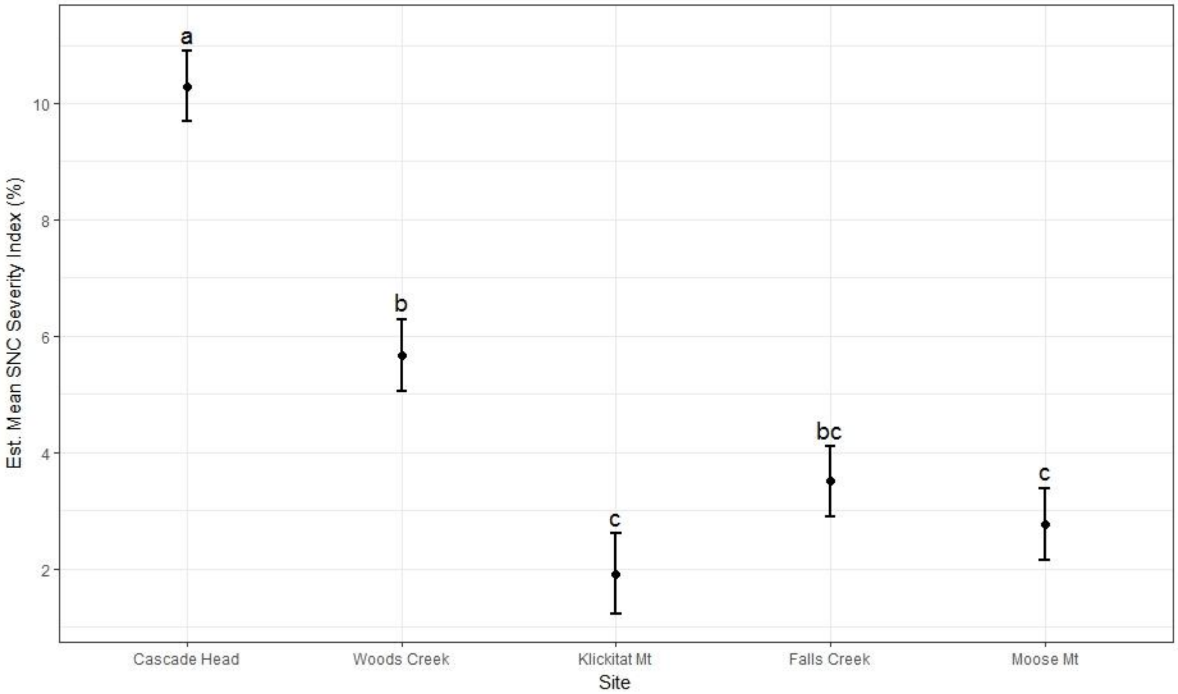

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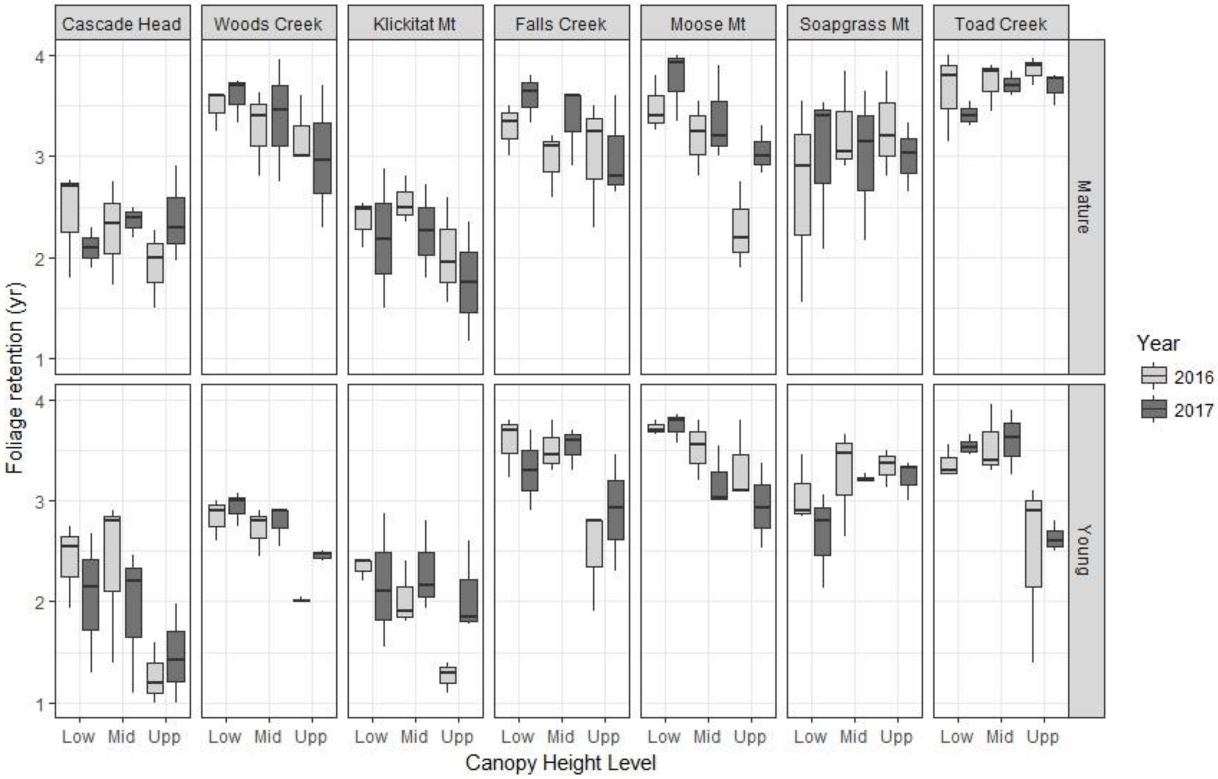

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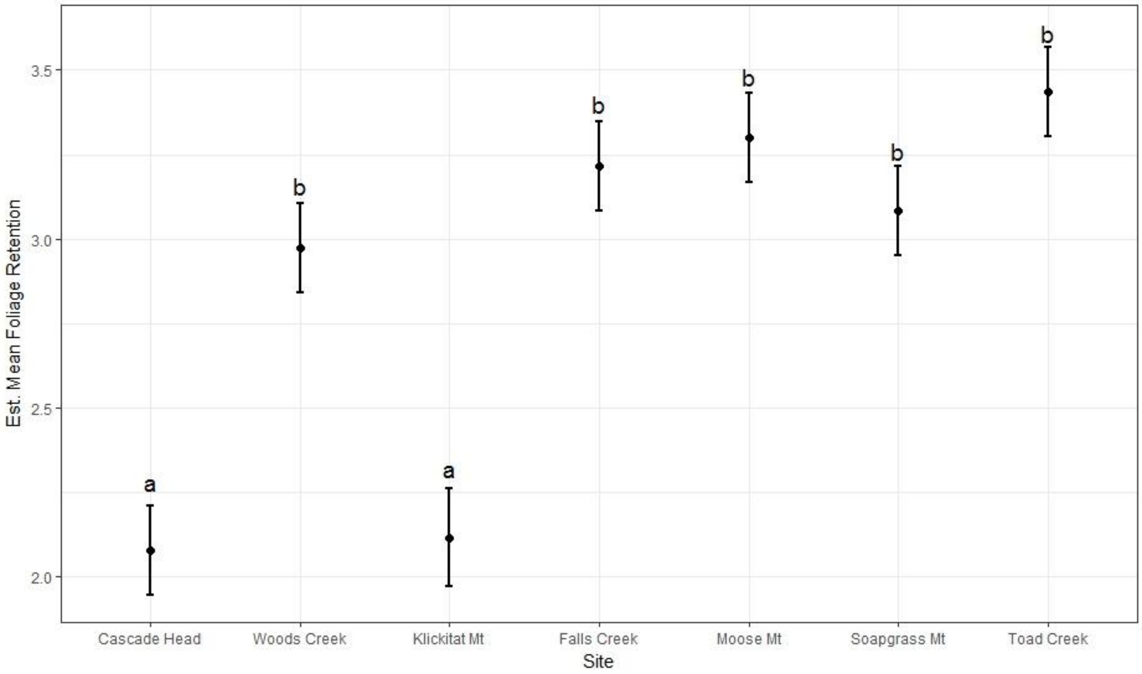

Figure 4.6. Estimation mean of foliage retention by site. Seven sites were present in MANOVA and mean comparison. The error bar represents mean ± 1standard error. Letters represents groups. The estimated mean of foliage retention between any two groups is statically different if the letters are different.

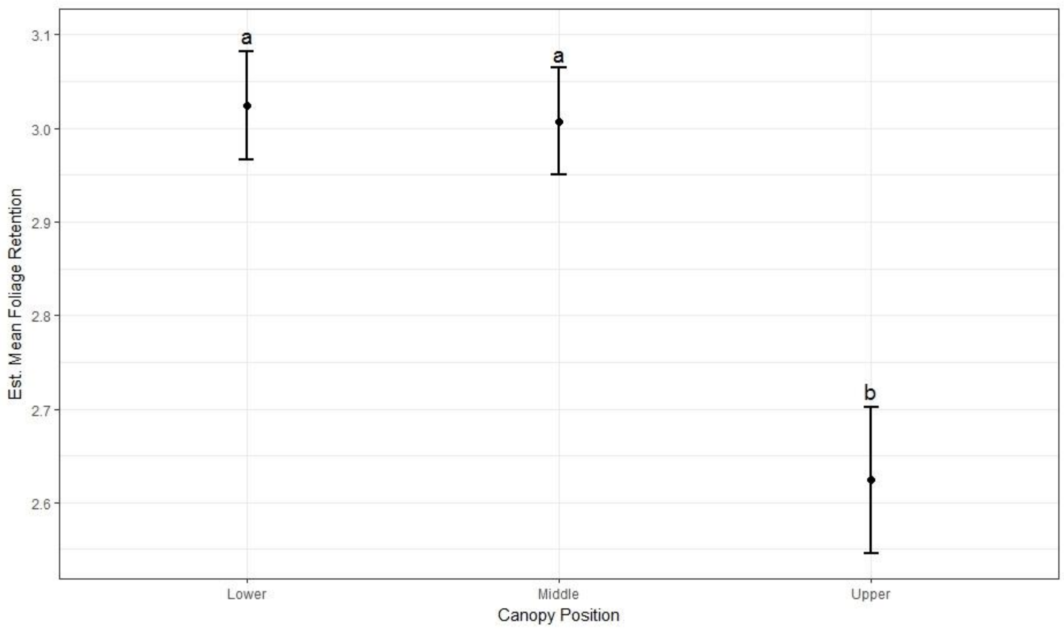

Figure 4.7. Estimation mean of foliage retention by canopy position. All sites were included in MANOVA and mean comparisons. The error bar represents mean ± 1standard error. Letters represents groups. The estimated mean of foliage retention between any two groups is statically different if the letters are different.

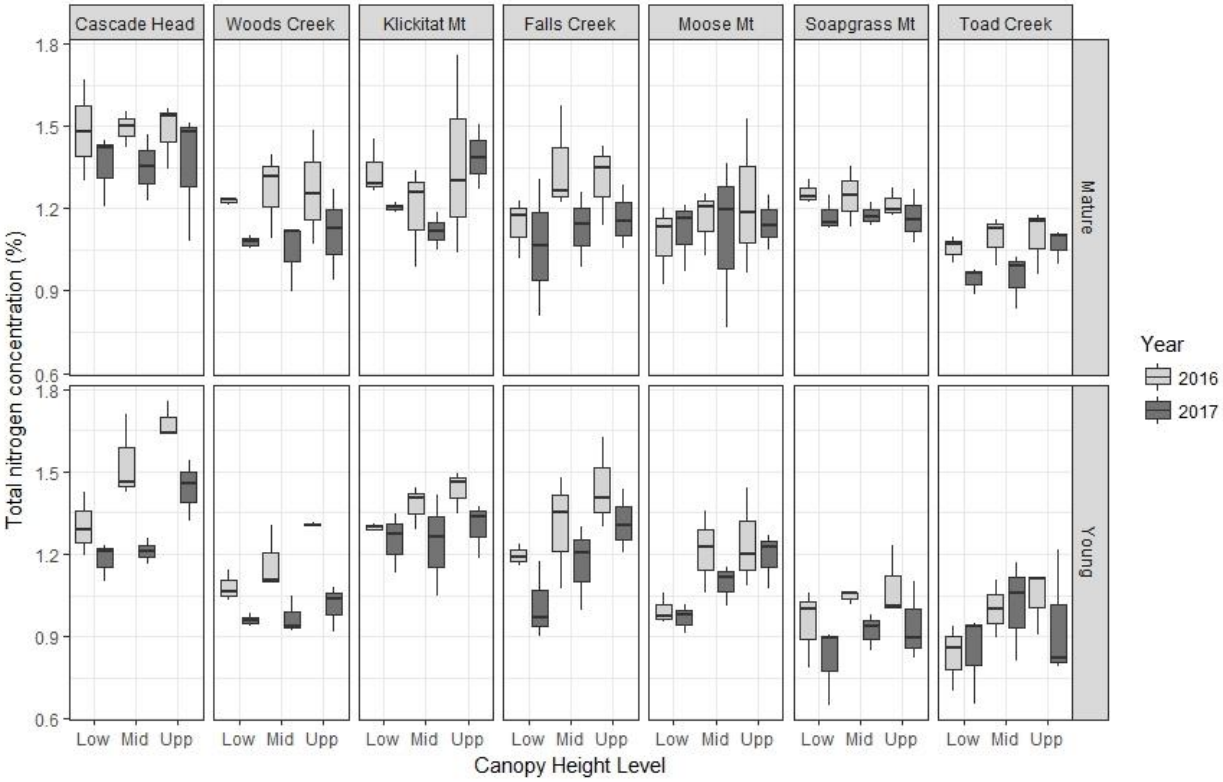

Figure 4.8. Total foliage nitrogen concentration at three canopy height levels among 7 sites in western Oregon in 2016-2017. Only 1-year-old needles were used for foliage nitrogen measurement. The whiskers represent the min and max values.

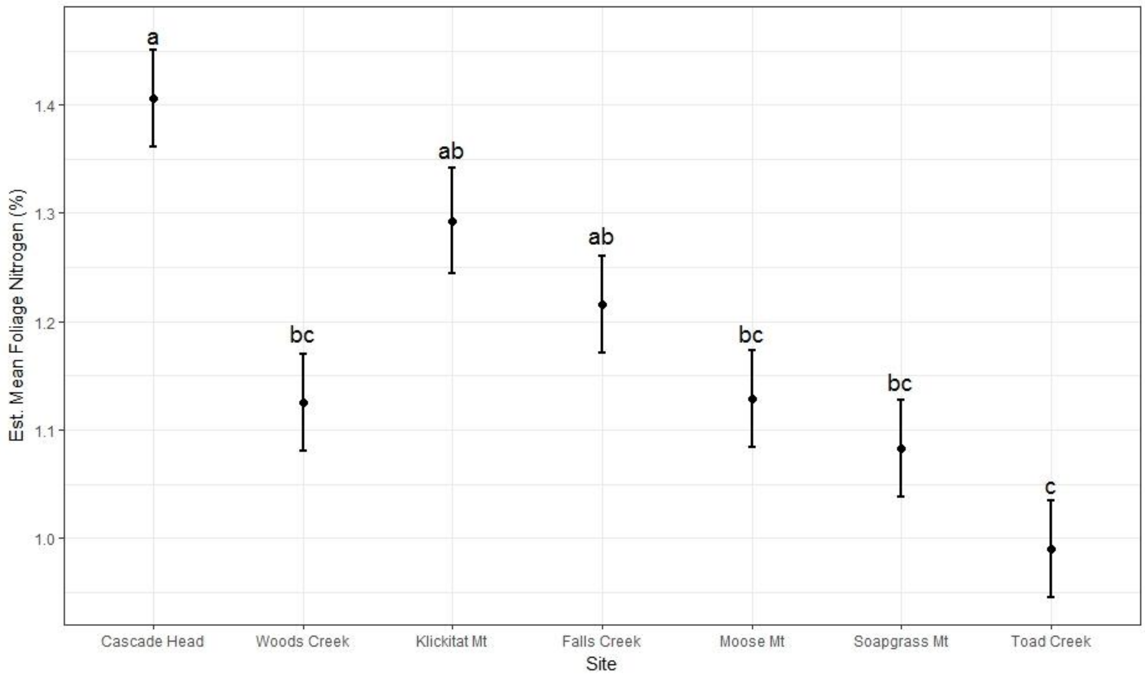

Figure 4.9. Estimation mean of foliage total nitrogen concentration by site. All seven sites were included in MANOVA and mean comparisons. The error bar represents mean ± 1standard error. Letters represents groups. The estimated mean of foliage nitrogen concentration between any two groups is statically different if the letters are different

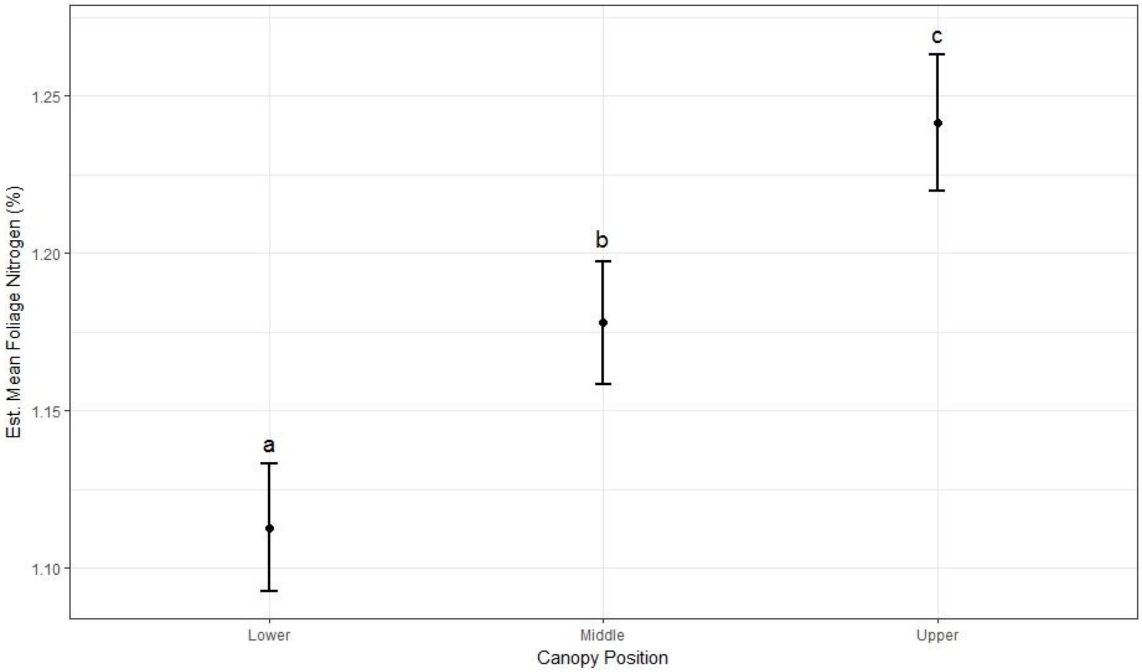

Figure 4.10. Estimation mean of foliage total nitrogen concentration by canopy considering site and year effects. All seven sites were included in MANOVA and mean comparisons. The error bar represents mean ± 1standard error. Letters represents groups. The estimated mean of foliage nitrogen between any two groups is statically different if the letters are different.

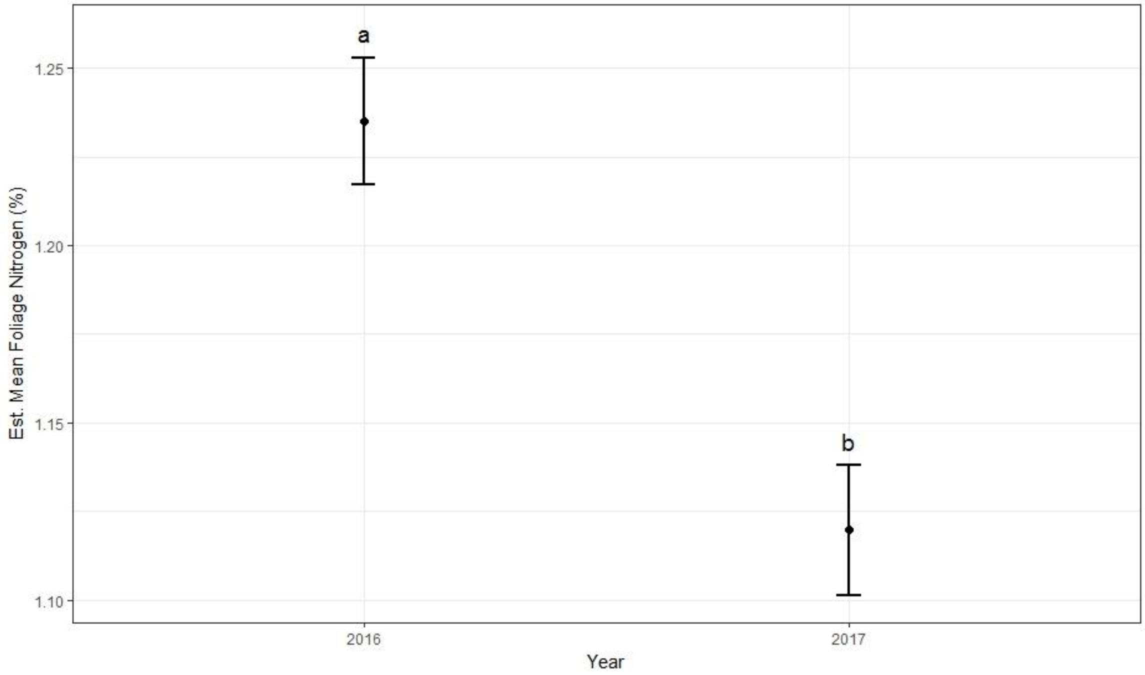

Figure 4.11. Estimation mean of foliage total nitrogen concentration by year considering canopy and site effects. All seven sites were included in MANOVA and mean comparisons. The error bar represents mean ± 1standard error. Letters represents groups. The estimated mean of foliage nitrogen between any two groups is statically different if the letters are different.

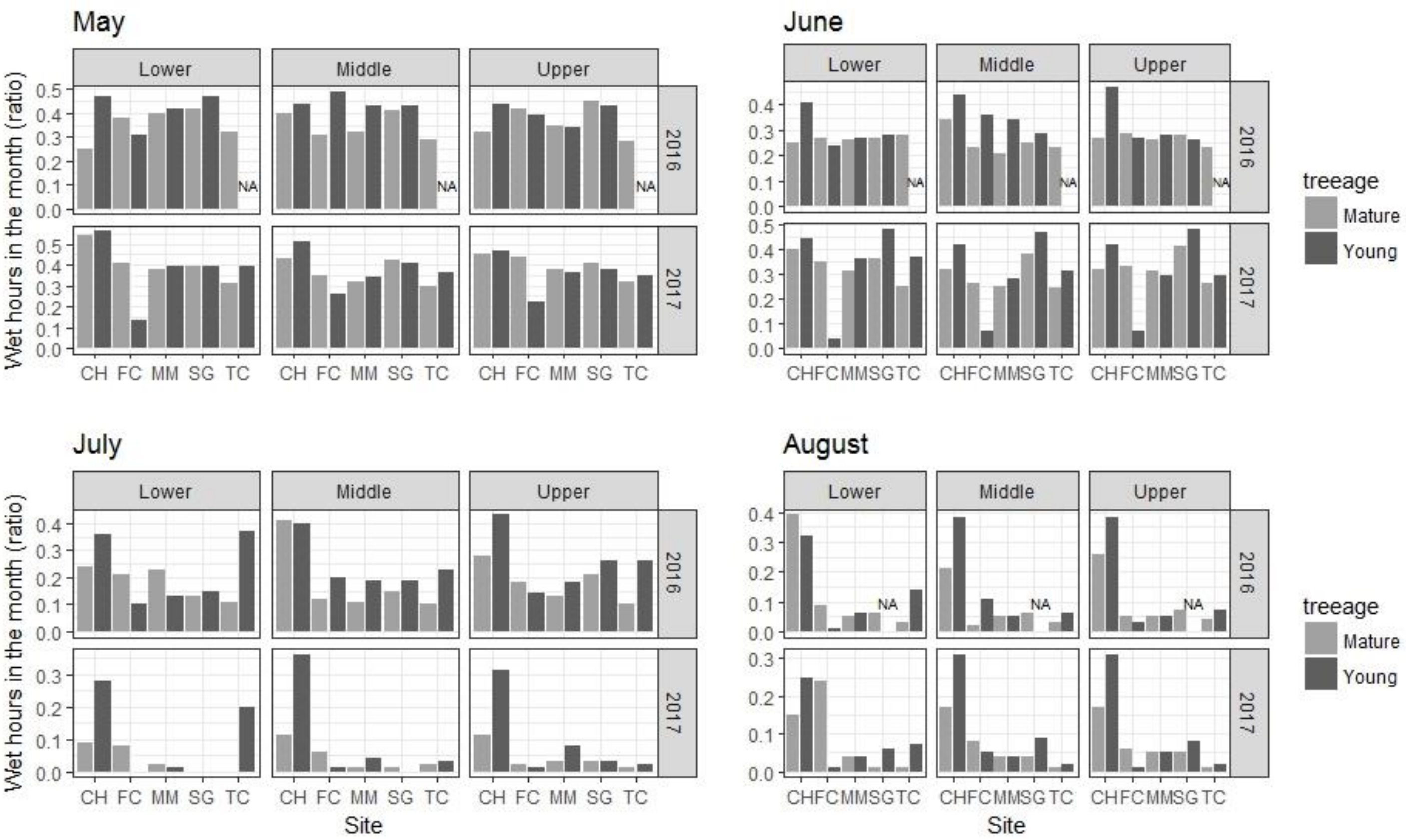

Figure 4.12. Wet hour ratio per month in Cascade Head (CH), Falls Creek (FC), Moose Mountain (MM), Soapgrass Mountain (SG), and Toad Creek (TC). We set up the wet/dry threshold as 280 mV based on the manual description as well as sensor performance on site, and counted it as a wet hour if the raw number >280mV. The leaf wetness duration was presented by counting the ratio of total wet hours per month / total hours per month. Due to technical issues of sensors, May 2016 and June 2016 in Toad Creek young plot, and August 2016 in Soapgrass Mountain young plot were missed in the figure and marked as “NA”.

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Table 4.1. Biophysical settings of the research sites. Including sites from Cascade Head, Woods Creek, and Klickitat Mountain in the Oregon Coast Range, and Moose Mountain, Falls Creek, Soapgrass Mountain, and Toad Creek in the western Cascade Mountains.

Site	Stand	Latutude	Longitude	Elev (m)	Tree age	Annual mean temp (°C)	Annual prep (mm)	Dec-Feb average temp(°C)	May-Aug prep(mm)
Cascade Head	Mature	45°02'26.82"	123°55'08.13"	147	~250	10.5	2517	5.9	301
	Young	45°02'14.75"	123°51'06.65"	171	~20	10.1	2760	5.4	310
Klickitat Mountain	Mature	44°14'38.03"	123°56'16.52"	383	150+	10.8	2198	5.9	247
	Young	44°14'10.89"	123°56'33.86"	610	10-15	10.4	2236	5.6	252
Woods Creek	Mature	44°32'00.67"	123°32'59.30"	523	120+	10.6	2353	4.7	183
	Young	44°32'12.13"	123°33'21.58"	496	6-8	10.7	2253	4.9	181
Moose Mountain	Mature	44°24'52.92"	122°23'39.48"	664	~120	10.9	1346	4.7	127
	Young	44°24'40.95"	122°23'52.26"	679	~10	9.7	1868	2.8	273
Falls Creek	Mature	44°23'44.24"	122°22'25.47"	556	~120	10.1	1922	2.9	276
	Young	44°23'42.38"	122°22'35.08"	562	15-20	10	1908	2.8	274
Soapgrass Mountain	Mature	44°20'52.67"	122°17'30.45"	1169	~450	7.9	2541	1.8	372
	Young	44°20'42.66"	122°17'38.10"	1193	10-15	8.2	2489	2.1	360
Toad Creek	Mature	44°25'32.86"	122°01'57.65"	1210	~280	7.4	2279	0.9	286
	Young	44°25'32.98"	122°02'19.68"	1193	8-10	7.4	2280	0.9	293

Table 4.2. Stand structure attributes of the 7 research sites where we conducted studies of SNC severity in western Oregon, 2016-2017.

		Trees per plot[3]		Trees per Ha		Average DBH (cm)		Basal Area (m^2/Ha)		Average Tree Height (m)	
Site	Plot	Douglas-fir	Other Trees	Douglas-fir	Other Trees	Douglas-fir	Other Trees	Douglas-fir	Other Trees	Douglas-fir	Other Trees
CH[1]	Mature	3	4	133	160	112.91	43.60	119.50	34.35	54.66	29.50
	Young	17	0	680	0	28.34	NA[2]	42.88	NA	20.00	NA
KT[1]	Mature	2	1	93	27	158.41	43.70	176.29	6.00	62.33	28.00
	Young	3	0	120	0	23.83	NA	5.35	NA	14.63	NA
WC[1]	Mature	2	8	93	307	107.03	21.24	68.30	10.99	57.72	NA
	Young	9	1	360	40	10.26	2.90	2.97	0.03	6.03	4.00
MM[1]	Mature	4	6	173	253	85.14	15.70	93.74	5.61	53.50	12.03
	Young	15	19	600	760	9.84	8.70	4.56	4.52	8.14	7.71
FC[1]	Mature	4	10	173	400	78.56	9.35	83.58	2.18	66.03	6.38
	Young	6	36	240	1440	24.40	4.03	11.22	1.83	10.58	5.94
SG[1]	Mature	2	5	67	213	156.44	42.63	133.18	30.81	54.12	21.39
	Young	10	8	400	320	15.48	14.85	7.52	5.54	9.41	8.83
TC[1]	Mature	4	13	173	533	81.44	18.33	88.41	13.77	45.73	11.52
	Young	4	32	160	1280	9.70	8.47	1.18	7.20	7.80	7.05

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

[2] NA means no data or not sufficient data to present.

[3] Dead trees and saplings are not included. For mature stand, we investigated three 8.9m radius plots centered with our sample tree, and averaged all 3 plots data to represent mature stand. For young stand, because the trees are closed to EPA weather station and the young trees grow evenly in stand, so we only investigated one 8.9m radius plot centered with EPA weather station.

Table 4.3. Results of preliminary MANOVA of SNC severity index, needle retention, and foliage nitrogen in study areas in western Oregon.

	SNC Severity Index (5 sites[2])			Needle retention (7 sites[3])			Foliage TN (7 sites[3])		
	F-value	p-value		F-value	p-value		F-value	p-value	
(Intercept)	295.9	< 0.001	***	4197.1	**< 0.001**	***	3705.1	**< 0.001**	***
Tree age	172.6	**< 0.001**	***	5.1	**0.033**	*	0.0	0.87	
Site	28.9	**< 0.001**	***	21.4	**< 0.001**	***	7.1	**0.001**	**
Tree age × Site	6.7	**0.002**	**	2	0.10	.	0.4	0.79	
Year	12.1	**0.003**	**	0.1	0.74		74.0	**< 0.001**	***
Tree age × Year	17.1	**0.001**	***	0	0.89		2.2	0.16	
Site × Year	10.9	**< 0.001**	***	0.6	0.74		3.6	**0.024**	*
Tree age × Site × Year	7.7	**0.001**	***	2.5	**0.046**	*	0.3	0.86	
Canopy	7.1	**0.006**	**	18.9	**< 0.001**	***	17.8	**0.0001**	***
Tree age × Canopy	1.5	0.26		3.4	**0.050**[1]	*	3.5	0.05	.
Site × Canopy	3.5	**0.004**	**	2	**0.050**[1]	*	0.9	0.52	
Tree age × Site × Canopy	2.1	0.06	.	1.4	0.18		1.3	0.29	
Year × Canopy	2.8	0.09	.	1.5	0.25		2.6	0.10	
Tree age × Year × Canopy	2.8	0.09	.	2.0	0.15		0.2	0.78	
Site × Year × Canopy	0.4	0.92		0.7	0.74		0.2	0.99	
Tree age × Site × Year × Canopy	0.3	0.94		1.5	0.16		0.8	0.64	

[1] The tree age x canopy and site x canopy interaction terms were not statistically significant (p-value = 0.19 and 0.50, respectively) when the needle retention data for Soapgrass Mountain and Toad Creek were excluded from the MANOVA, indicating that canopy differences at the five lower elevation sites were similar but different than at the higher elevation sites.

[2] Including sites Cascade Head, Woods Creek, and Klickitat Mountain, Moose Mountain, and Falls Creek. Soapgrass Mountain and Toad Creek were excluded because almost all SNC values from those sites were zeros.

[3] Including sites Cascade Head, Woods Creek, and Klickitat Mountain, Moose Mountain, Falls Creek, Soapgrass Mountain, and Toad Creek.

Table 4.4. Results of MANOVA by individual sites. Because site is a crucial factor involved in most interactions, the MANOVA was re-run by site to clarify the effects from other factors. Tree age was another key factor in SNC severity index analysis and involved in many interactions. Canopy position was contributed in needle retention when considering the site effect.

		SNC Severity Index			Needle retention		
		F-value	p-value		F-value	p-value	
CH[1]	(Intercept)	108.6	**< 0.001**	***	129.8	**< 0.001**	***
	Tree age	26.0	**0.007**	**	0.8	0.413	
	Year	6.0	0.070	.	0.4	0.567	
	Tree age × Year	0.8	0.413		2.0	0.229	
	Canopy	20.9	**0.017**	*	4.1	0.137	
	Tree age × Canopy	5.3	0.105		2.2	0.256	
	Year × Canopy	5.5	0.100	.	6.3	0.084	.
	Tree age × Year × Canopy	4.9	0.115		0.8	0.519	
KT[1]	(Intercept)	47.8	**0.006**	**	143.1	**0.001**	**
	Tree age	35.6	**0.009**	**	0.4	0.596	
	Year	45.9	**0.007**	**	0.0	0.938	
	Tree age × Year	44.1	**0.007**	**	2.0	0.252	
	Canopy	0.0	0.988		338.3	**0.003**	**
	Tree age × Canopy	0.1	0.948		46.4	**0.021**	*
	Year × Canopy	0.2	0.864		1.8	0.361	
	Tree age × Year × Canopy	0.3	0.783		7.4	0.120	
WC[1]	(Intercept)	138.0	**< 0.001**	***	1739.1	1.976	***
	Tree age	136.7	**< 0.001**	***	23.9	**0.008**	**
	Year	17.5	**0.014**	*	0.4	0.549	
	Tree age × Year	17.3	**0.014**	*	0.4	0.566	
	Canopy	14.6	**0.028**	*	9.3	**0.052**	.*
	Tree age × Canopy	15.0	**0.027**	*	0.9	0.498	
	Year × Canopy	3.7	0.154		0.0	0.996	
	Tree age × Year × Canopy	3.8	0.151		0.7	0.577	

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

Table 4.4. (Continued)

		SNC Severity Index			Needle retention		
		F-value	p-value		F-value	p-value	
MM[1]	(Intercept)	18.8	**0.012**	**	1679.5	**< 0.001**	***
	Tree age	17.12	**0.014**	**	1.8	0.251	
	Year	284.6	**< 0.001**	***	0.9	0.392	
	Tree age × Year	258.5	**< 0.001**	***	12.9	**0.023**	*
	Canopy	4.8	0.117		257.3	**< 0.001**	***
	Tree age × Canopy	3.9	0.148		5.3	0.105	
	Year × Canopy	1.4	0.379		1.5	0.349	
	Tree age × Year × Canopy	0.9	0.490		1.3	0.396	
FC[1]	(Intercept)	100.1	**0.001**	***	1499.2	**< 0.001**	***
	Tree age	98.0	**0.001**	***	0.0	0.942	
	Year	0.4	0.565		4.4	0.105	
	Tree age × Year	0.4	0.585		2.0	0.227	
	Canopy	4.2	0.134		4.6	0.122	
	Tree age × Canopy	4.5	0.127		4.9	0.114	
	Year × Canopy	42.8	**0.006**	**	0.3	0.766	
	Tree age × Year × Canopy	34.4	**0.009**	**	0.8	0.528	
SG[1]	(Intercept)	-	-		747.3	**< 0.001**	***
	Tree age	-	-		0.2	0.700	
	Year	-	-		1.4	0.310	
	Tree age × Year	-	-		0.2	0.651	
	Canopy	-	-		1.5	0.358	
	Tree age × Canopy	-	-		0.0	0.980	
	Year × Canopy	-	-		0.2	0.869	
	Tree age × Year × Canopy	-	-		1.1	0.443	
TC[1]	(Intercept)	-	-		1670.4	**< 0.001**	***
	Tree age	-	-		8.3	**0.045**	*
	Year	-	-		0.0	0.968	
	Tree age × Year	-	-		1.1	0.361	
	Canopy	-	-		14.6	**0.029**	*
	Tree age × Canopy	-	-		13.4	**0.032**	*
	Year × Canopy	-	-		0.0	0.972	
	Tree age × Year × Canopy	-	-		0.4	0.687	

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

Appendix

Appendix I. Summary of SNC incidence for all needle age classes in 2016. The number in front of ± is average of three trees and the number followed is standard deviation.

2016			yr1	yr2	yr3	yr4	yr5	yr6	yr7	yr8	yr9	yr10
CH[1]	Mature	Upper	10.00 ± 7.21	88.67 ± 10.07	67.21 ± 46.78	80.00 ± 2.83	63.67 ± 42.90	86.00 ± NA	-	-	-	-
		Middle	4.67 ± 8.08	98.00 ± 0.00	96.00 ± 5.29	85.23 ± 13.45	96.06 ± 2.74	-	-	-	-	-
		Lower	1.33 ± 2.31	88.67 ± 2.31	96.00 ± 6.93	69.33 ± 4.62	90.20 ± 5.39	81.82 ± NA	-	-	-	-
	Young	Upper	95.33 ± 4.16	99.33 ± 1.15	-	-	-	-	-	-	-	-
		Middle	68.67 ± 23.86	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-	-
		Lower	8.00 ± 8.72	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-
KT[1]	Mature	Upper	2.67 ± 3.06	34.67 ± 37.65	60.67 ± 37.22	65.75 ± 19.44	12.00 ± NA	-	-	-	-	-
		Middle	0.00 ± 0.00	24.67 ± 15.01	74.67 ± 13.32	27.63 ± 17.45	17.55 ± 17.29	34.07 ± 25.36	10.88 ± 1.24	7.69 ± NA	-	-
		Lower	0.67 ± 1.15	20.00 ± 12.49	74.00 ± 5.29	29.41 ± 11.94	17.00 ± 9.90	12.53 ± 9.23	0.00 ± NA	-	-	-
	Young	Upper	14.00 ± 12.49	96.67 ± 4.16	-	-	-	-	-	-	-	-
		Middle	15.33 ± 8.33	96.00 ± 6.93	100 ± NA	100 ± NA	-	-	-	-	-	-
		Lower	2.00 ± 3.46	96.00 ±5.29	92.67 ± 9.45	89.33 ± 16.77	99.00 ± 1.41	-	-	-	-	-
WC[1]	Mature	Upper	0.00 ± 0.00	0.67 ± 1.15	6.00 ± 8.72	22.00 ± 18.33	5.33 ± 5.03	-	-	-	-	-
		Middle	0.00 ± 0.00	1.33 ± 2.31	29.33 ± 10.07	26.67 ± 11.02	7.03 ± 2.68	6.00 ± 2.83	4.26 ± NA	0.00 ± NA	4.00 ± NA	0.00 ± NA
		Lower	0.00 ± 0.00	4.67 ± 3.06	55.33 ± 11.02	52.67 ± 2.31	12.00 ± 5.29	14.67 ± 7.57	12.00 ± 10.58	2.00 ± 2.83	0.00 ± NA	-
	Young	Upper	0.67 ± 1.15	85.33 ± 13.32	-	-	-	-	-	-	-	-
		Middle	0.00 ± 0.00	99.33 ± 1.15	99.33 ± 1.15	61.00 ± 26.87	-	-	-	-	-	-
		Lower	0.67 ± 1.15	100.00 ± 0.00	100.00 ± 0.00	96.00 ± 6.93	-	-	-	-	-	-

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

[2] NA means there was only one sample so it lacked of standard deviation.

[3] "-" means sample wasn't present.

Appendix I. (Continued)

2016			yr1	yr2	yr3	yr4	yr5	yr6	yr7	yr8	yr9	yr10
MM[1]	Mature	Upper	0.67 ± 1.15	17.33 ± 21.57	27.33 ± 29.69	28.04 ± 36.71	63.16 ± NA	-	-	-	-	-
		Middle	5.33 ± 6.11	25.33 ± 33.72	66.67 ± 52.62	63.33 ± 41.05	55.00 ± 52.33	50.00 ± 59.40	20.00 ± NA	10.00 ± NA	38.46 ± NA	-
		Lower	8.67 ± 8.33	24.67 ± 9.45	70.67 ± 40.61	58.00 ± 33.65	44.00 ± 22.63	29.00 ± 21.21	12.00 ± NA	20.00 ± NA		-
	Young	Upper	20.00 ± 3.46	92.67 ± 6.43	100.00 ± 0.00	91.00 ± 12.73	-	-	-	-	-	-
		Middle	17.33 ± 3.06	98.67 ± 2.31	100.00 ± 0.00	97.33 ± 4.62	95.33 ± 8.08	-	-	-	-	-
		Lower	24.00 ± 6.93	99.33 ± 1.15	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	86.37 ± 19.28	100.00 ± NA	-	-	-
FC[1]	Mature	Upper	4.67 ± 3.06	4.00 ± 2.00	40.00 ± 45.74	42.53 ± 22.17	27.00 ± 7.07	18.37 ± NA	-	-	-	-
		Middle	2.00 ± 2.00	6.67 ± 6.43	78.67 ± 22.03	48.00 ± 21.63	59.51 ± 8.04	40.00 ± 5.66	-	-	-	-
		Lower	8.67 ± 10.26	12.67 ± 9.24	69.33 ± 9.45	64.67 ± 20.82	73.33 ± 22.12	51.52 ± 32.13	40.23 ± 6.39	48.87 ± 33.75	-	-
	Young	Upper	12.00 ± 12.49	98.00 ± 2.00	97.44 ± 4.44	-	-	-	-	-	-	-
		Middle	19.33 ± 5.77	100.00 ± 0.00	98.67 ± 2.31	100.00 ± 0.00	99.33 ± 1.15	97.83 ± NA	-	-	-	-
		Lower	38.00 ± 13.11	98.67 ± 2.31	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-
SG[1]	Mature	Upper	0.00 ± 0.00	2.00 ± 2.00	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± NA	0.00 ± NA		-
		Middle	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	2.94 ± 4.16	0.00 ± NA	-
		Lower	1.33 ± 2.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Young	Upper	0.00 ± 0.00	0.00 ± 0.00	2.67 ± 4.62	2.67 ± 1.15	2.00 ± 2.83	0.00 ± NA	-	-	-	-
		Middle	0.00 ± 0.00	0.67 ± 1.15	8.67 ± 2.31	8.67 ± 3.06	12.67 ± 18.48	4.67 ± 8.08	4.00 ± 5.66	0.00 ± NA	-	-
		Lower	0.00 ± 0.00	4.00 ± 4.00	46.67 ± 40.41	39.33 ± 27.23	34.67 ± 16.17	34.00 ± 51.96	28.67 ± 42.91	13.83 ± 17.86	90.00 ± NA	-
TC[1]	Mature	Upper	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± NA	0.00 ± NA
		Middle	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Lower	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Young	Upper	0.67 ± 1.15	2.67 ± 3.06	2.00 ± 2.83	2.00 ± 2.83	-	-	-	-	-	-
		Middle	3.33 ± 4.16	3.33 ± 4.16	2.00 ± 2.00	2.67 ± 3.06	1.00 ± 1.41	6.00 ± NA	2.00 ± NA	2.94 ± NA	-	-
		Lower	8.00 ± 3.46	1.33 ± 1.15	2.00 ± 2.00	2.00 ± 2.00	1.33 ± 2.31	0.67 ± 1.15	0.00 ± 0.00	-	-	-

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

[2] NA means there was only one sample so it lacked of standard deviation.

[3] "-" means sample wasn't present.

Appendix II. Summary of SNC incidence for all needle age classes in 2017. The number in front of ± is average of three trees and the number followed is standard deviation.

2017			yr1	yr2	yr3	yr4	yr5	yr6	yr7	yr8	yr9	yr10
CH[1]	Mature	Upper	40.67 ± 26.56	96.67 ± 5.77	80.67 ± 26.86	100.00 ± NA	100.00 ± NA	100.00 ± NA	-	-	-	-
		Middle	35.33 ± 17.47	99.33 ± 1.15	98.67 ± 1.15	98.67 ± 2.31	100.00 ± NA	-	-	-	-	-
		Lower	12.67 ± 6.11	97.33 ± 4.62	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-	-
	Young	Upper	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-	-	-	-	-	-	-
		Middle	80.67 ± 19.22	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-
		Lower	45.33 ± 24.85	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-
KT[1]	Mature	Upper	0.00 ± 0.00	12.00 ±0.00	28.92 ± 12.61	41.67 ± 58.92	-	-	-	-	-	-
		Middle	2.00 ± 2.83	20.00 ± 28.28	44.00 ± 22.63	44.00 ± 16.97	26.95 ± 23.97	0.00 ± NA	7.14 ± NA	6.82 ± NA	-	-
		Lower	3.00 ± 1.41	3.00 ± 1.41	28.00 ± 16.97	57.00 ± 1.41	28.67 ± 6.60	29.00 ± 29.70	10.00 ± NA	6.25 ± NA	-	-
	Young	Upper	8.67 ± 2.31	91.33 ± 8.33	99.33 ± 1.15	100.00 ± NA	-	-	-	-	-	-
		Middle	6.67 ± 5.77	85.33 ± 6.11	98.00 ± 2.00	97.44 ± 4.44	97.22 ± 4.81	100.00 ± NA	-	-	-	-
		Lower	13.33 ± 6.43	80.67 ± 20.03	95.33 ± 8.08	100.00 ± 0.00	95.87 ± 2.02	-	-	-	-	-
WC[1]	Mature	Upper	0.00 ± 0.00	0.67 ± 1.15	1.33 ± 1.15	4.67 ± 4.62	20.00 ± 14.00	0.00 ± 0.00	-	-	-	-
		Middle	0.00 ± 0.00	1.33 ± 2.31	6.67 ± 6.43	32.00 ± 0.00	23.33 ± 8.08	6.78 ± 1.35	7.43 ± 6.46	10.00 ± NA	-	-
		Lower	1.33 ± 1.15	2.00 ± 2.00	17.33 ± 7.02	30.67 ± 8.08	40.00 ± 5.29	10.00 ± 9.17	15.33 ± 7.57	11.52 ± 6.12	1.55 ± 2.68	-
	Young	Upper	0.67 ± 1.15	30.00 ± 27.78	80.00 ± 23.58	-	-	-	-	-	-	-
		Middle	2.00 ± 2.00	50.67 ± 18.90	94.00 ± 8.72	94.00 ± 10.39	-	-	-	-	-	-
		Lower	2.00 ± 3.46	52.67 ± 27.30	99.33 ± 1.15	98.67 ± 1.15	57.89 ± NA	-	-	-	-	-

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

[2] NA means there was only one sample so it lacked of standard deviation.

[3] "-" means sample wasn't present.

Appendix II. (Continued)

2017			yr1	yr2	yr3	yr4	yr5	yr6	yr7	yr8	yr9	yr10
MM[1]	Mature	Upper	0.00 ± 0.00	3.33 ± 4.16	16.00 ± 19.70	31.38 ± 23.74	29.41 ± NA	-	-	-	-	-
		Middle	0.67 ± 1.15	0.67 ± 1.15	34.67 ± 30.35	51.85 ± 40.13	26.79 ± 6.77	18.00 ± NA	2.00 ± NA	11.76 ± NA	6.45 ± NA	-
		Lower	0.67 ± 1.15	1.33 ± 2.31	52.67 ± 44.60	56.00 ± 49.76	53.33 ± 47.60	33.00 ± 38.18	25.00 ± 32.53	0.00 ± NA	6.82 ± NA	-
	Young	Upper	0.00 ± 0.00	60.00 ± 40.15	89.94 ± 14.11	83.50 ± 12.02	-	-	-	-	-	-
		Middle	1.33 ± 2.31	67.33 ± 31.13	99.33 ± 1.15	96.00 ± 5.66	100.00 ± NA	-	-	-	-	-
		Lower	3.33 ± 1.15	78.67 ± 25.79	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-	-	-	-
FC[1]	Mature	Upper	0.00 ± 0.00	2.00 ± 2.00	33.33 ± 20.43	40.04 ± 26.99	60.00 ± NA	38.00 ± NA	40.43 ± NA	-	-	-
		Middle	0.00 ± 0.00	3.33 ± 4.16	34.67 ± 31.13	68.67 ± 39.00	55.33 ± 7.57	57.74 ± 20.17	50.23 ± 16.65	-	-	-
		Lower	0.00 ± 0.00	2.00 ± 2.00	52.67 ± 17.24	88.67 ± 9.87	72.98 ± 18.35	74.00 ± 21.17	39.88 ± 20.67	-	-	-
	Young	Upper	36.00 ± 52.12	97.33 ± 3.06	98.67 ± 2.31	96.00 ± NA	100 ± NA	-	-	-	-	-
		Middle	22.00 ± 29.60	99.33 ± 1.15	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-	-	-	-
		Lower	32.00 ± 43.41	95.33 ± 5.03	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± NA	-	-	-	-
SG[1]	Mature	Upper	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.19 ± 5.19	2.39 ± 0.55	0.00 ± NA	-	-	-
		Middle	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 1.15	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± NA	0.00 ± NA	0.00 ± NA
		Lower	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Young	Upper	0.00 ± 0.00	0.00 ± 0.00	1.33 ± 2.31	2.67 ± 3.06	7.00 ± 4.24	4.00 ± NA	3.33 ± NA	-	-	-
		Middle	0.67 ± 1.15	0.00 ± 0.00	19.33 ± 18.04	18.67 ± 20.43	13.33 ± 8.08	10.00 ± 5.29	4.00 ± 5.66	4.00 ± 5.66	-	-
		Lower	1.33 ± 2.31	4.00 ± 5.29	31.33 ± 24.68	43.33 ± 44.02	40.67 ± 38.02	44.42 ± 37.50	58.00 ± 59.40	75.00 ± NA	19.23 ± NA	-
TC[1]	Mature	Upper	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± NA	-
		Middle	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Lower	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Young	Upper	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-	-	-	-	-	-
		Middle	0.00 ± 0.00	0.67 ± 1.15	0.67 ± 1.15	0.67 ± 1.15	2.00 ± 2.00	0.00 ± 0.00	-	-	-	-
		Lower	0.00 ± 0.00	0.67 ± 1.15	1.33 ± 2.31	0.67 ± 1.15	2.67 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± NA	-	-

[1] The abbreviation of study sites is Cascade Head (CH), Woods Creek (WC), and Klickitat Mountain (KT), Moose Mountain (MM), Falls Creek (FC), Soapgrass Mountain (SG), and Toad Creek (TC).

[2] NA means there was only one sample so it lacked of standard deviation.

[3] "-" means sample wasn't present.

CHAPTER 5 – GENERAL CONCLUSION

Swiss needle cast (SNC) is a widespread foliage disease of Douglas-fir (*Pseudotsuga menziesii*). It is caused by the native and ever-present endophyte fungus *Nothophaeocryptopus gaeumannii*. The reproductive structure of *N. gaeumannii*, pseudothecia, blocks stomates on needles, thereby physically reducing photosynthetic rates and productivity of Douglas-fir without cell damage of needles (Manter et al. 2000). It generally does not cause tree death in most infected areas. However, SNC currently is causing large growth loss in forests across coastal Oregon and Washington states and is thought to be one of the largest threats to Douglas-fir plantations. More than 238,705 ha of plantation was infected in 2015 (Ritóková et al. 2016) in this region. Defoliation may occur if 50% of the needle stomates are occluded by the fungus (Hansen et al. 2000). For the heavily infected region of NW Oregon, wood volume losses can be more than 50% (Maguire et al. 2011).

The impact of SNC on Douglas-fir can be visible. In Figure 5.1, the heavily infected tree has few needles on its twigs in later spring, while the healthy tree has at least 3-4 year-old needles on twigs. Because severe SNC infection causes loss of needles, the needle retention of Douglas-fir can be representative of SNC disease severity. Specifically, needles from middle canopy have better correlation with SNC severity and needle retention than the needles from lower and upper canopy (Fig. 5.2).

Foliar nutrients are mostly positively associated with SNC disease severity. In chapter 2 we saw that nitrogen, carbon, Na, K, and S are positively correlated to SNC disease severity. It is not known why the foliar nutrients are associated with SNC severity, and the cause-effect relationship between SNC and foliar nutrients has not been studied. The mobility of nutrients might be related to patterns we observed. For example, nitrogen is a highly mobile element in needles, it can be re-translocated to younger cohorts. More studies are needed but defoliation by SNC infection might cause nitrogen to be re-located to current needles with less occlusion on stomates.

Climate is considered another key factor related to SNC disease severity, especially winter temperature and summer precipitation (Manter et al. 2005). In Chapter 3, we saw that mean temperature in late fall, winter, and early spring is associated to SNC disease severity. Also, dew point temperature becomes a crucial factor because all-year-round monthly data are associated with SNC disease severity. Summer precipitation is reported as a driving factor for SNC (Lee et al. 2013), although the monthly data in summer did not show much association with SNC.

Tillamook is one of the most severely impacted areas of the SNC epidemic region (Hansen et al. 2000). Data from Swiss Needle Cast Cooperative research and monitoring plot network showed the association between SNC disease severity and climate (e.g. monthly mean temperature and dew point temperature) was much different in Tillamook in comparison to other regions.

Although well-studied in young Douglas-fir plantation in Oregon and Washington coastal area, SNC data from mature and old-growth forests are rare. Therefore, SNC disease ecology is not well understood in late-successional forests. Based on both anecdotal observations and investigation in Chapter 5, SNC disease severity in mature forests is apparently lower than in young forests, even when the mature and young trees are in the same region (Fig. 5.3). Although the needle retention is lower in coastal area than in inland region, only Cascade Head (the plot closest to the ocean) is considered in the core epidemic area.

Needle retention, foliage nitrogen, and leaf wetness data were studied in Chapter 4, but did not provide strong evidence for why the fungus infection is less on mature trees than on young trees. Temperature difference caused by forest structure might be one of the reason, but this requires more study in order to understand the ecology of fungus in mature forest. Additionally, because needle morphology (Apple et al. 2000) and plant defensive mechanism (Erwin et al. 2001) are different on mature and young trees, the physiological and chemical characteristics of needles might be other potential reasons associated with the difference of SNC severity between mature and young trees.

It is common practice to use 2-year-old needles as material for SNC disease severity (Manter et al. 2005). However, *N. gaeumannii* has more presence on 3-5 year old needles than 2-year-old needles of mature and old-growth trees, suggesting that 2-year-old needles might not be a good indicator for mature trees, especially mature trees on the west slope of the Cascade Mountain Range. More studies are needed to confirm which needle cohort is the best for SNC severity indicator on mature Douglas-fir.

Douglas-fir is the most valuable timber species in North America (OFRI, 2017). In Oregon, it accounts for ~17% of the U.S. softwood lumber output by Oregon Forest Resources Institute in 2015, related to 5.2 billion board feet wood volume and ~$2.3 billion dollars annually. The dominant softwood species in Oregon is Douglas-fir. Although SNC may not cause tree death in most situations, the loss of annual volume growth is significant. If maximizing profit is important for land owners, then land owners need to manage Douglas-fir plantations with SNC to reduce their timber and economic losses.

Modeling shows that climate the Pacific Northwest is getting warmer and drier (Miles et al. 2000). However, seasonality will be very important for foliage diseases, especially June and July leaf wetness factors and fall, winter and spring temperatures. Scientists are particularly worried that climate change may drive more SNC in the Pacific Northwest due to the warmer winters (Lee et al. 2016), which could in turn affect the higher elevation Douglas-fir plantations in the Cascade Mountains.

Lee et al. (2017) has shown growth suppression attributed to SNC in old trees. In Tillamook region, even mature Douglas-fir forests (~80 year old) are heavily suppressed by SNC, affecting the canopy structure of mature and old-growth forests (Fig. 5.4). The mature and old-growth forests are associated with higher biodiversity than the young forests, and provide habitats for vegetation, invertebrates and birds, and mammals. Old-growth is associated with some endangered species, like spotted owl (*Strix occidentalis*) and marbled murrelet (*Brachyramphus marmoratus*). If SNC affects old-growth trees and makes trees weaker or hazardous, wildlife habitats may be affected.

There are still many questions about SNC for both young plantation and mature forests. For plantation management, we need more knowledge on the cause-effect of nutrients in foliage and especially climate variable such a dew. This may help with silviculture strategies. If the weather becomes warmer in North America, it is also important to think about how to reduce the disease impacts, which would require further studies. On the other hand, for mature and old-growth Douglas-fir, more studies are needed to answer questions about fungus-host relationship. How is the fungal ecology in mature and old-growth Douglas-fir different from young stands? Does it require more time for maturation of the reproductive structures? How big are the impacts that could happen on mature trees? If tree growth is suppressed by foliage disease, how long would the tree be more vulnerable to other pathogens or weather damage (e.g. wind break and snow pack)? Could SNC be related to forest decline, and therefore change wildlife habitat and affect endangered species in Pacific Northwest?

For further research, I suggest examination of detailed study of nutrients in foliage, and how these interact with host and pathogen, in addition, does SNC severity induce foliage nutrients to relocate to younger needles. In Chapter 2 and Chapter 3, we narrowed down some factors related to SNC severity and, the results can be used for building more complicated models including foliar nutrients and climate variables, to know which factors contribute more to SNC on Oregon coastal Douglas-fir plantations. With regard to mature and old-growth Douglas-fir forests, more studies on 3-5-year-old needles are needed for determine the best cohort for SNC severity index. It is also important to have more research in epidemic mature forests (i.e. Tillamook), to understand the impacts of SNC on mature and old-growth Douglas-fir.

In 2015, the Paris Agreement was adopted by the United Nations Framework Convention on Climate Change (UNFCCC) members to response to global warming. Carbon accumulation, or “carbon tax” is considered a way to contribute the CO_2 emission control. Douglas-fir is a valuable, fast-growing coniferous species in North America. If SNC is more severe due to the climate change, the suppression of Douglas-fir growth may cause less carbon fixation from the air. Reduced carbon sequestration in young and old forests is likely, but there may also be habitat impacts that are not being quantified. Given that importance of growing Douglas-fir and the native foliage disease, SNC may have more impacts from climate change than we thought.

My hope is to contribute more ecological research of old-growth forest canopies, and lead some cross-continent collaborations between Pacific Northwest and mountain areas in Taiwan. The species of conifers are similar in both regions, such as cedar, cypress, spruce, hemlock, true firs, and Taiwan and North America even share a *Pseudotsuga* species, the genius of Douglas-fir. Pathological study on coniferous species in Taiwan is rare, and cross country collaboration might provide broader perspectives for further ecological research.

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Lists of Figure

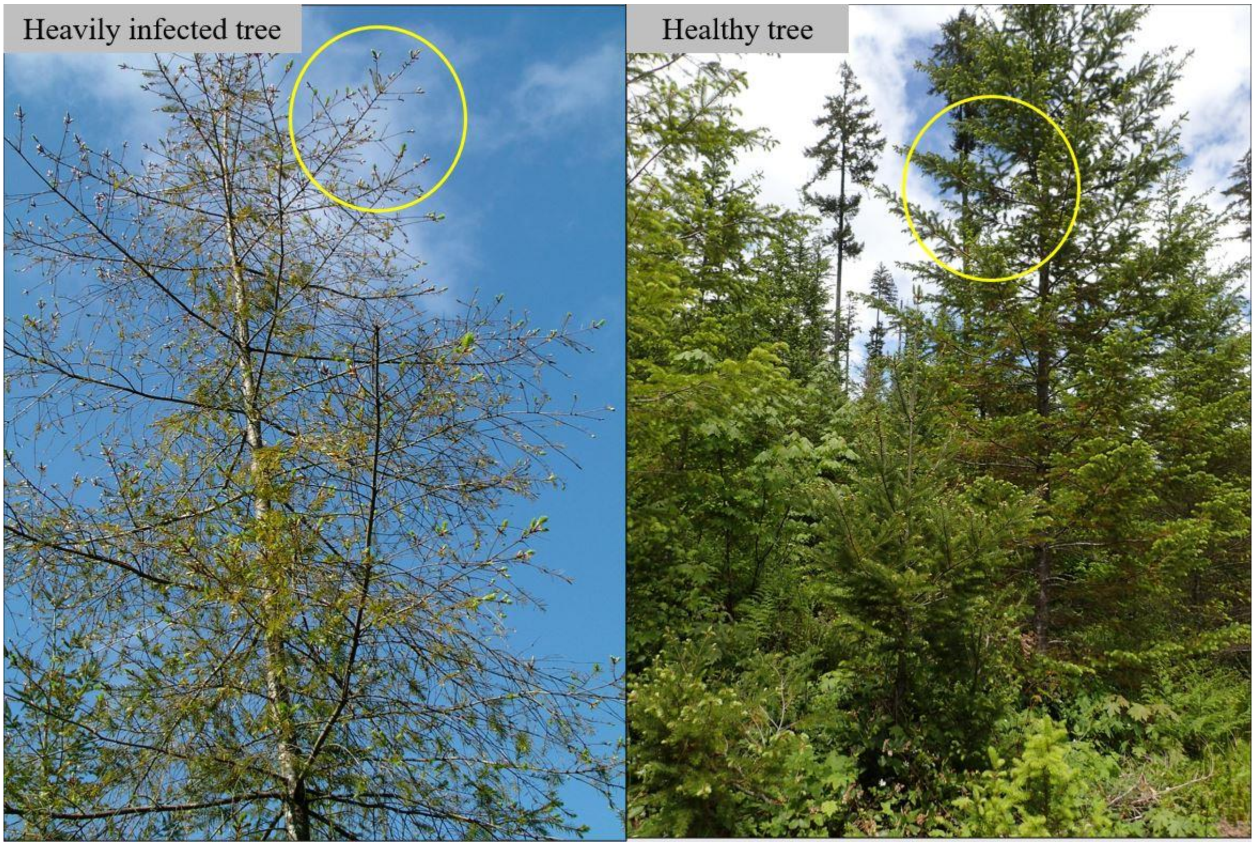

Figure 5.1. Douglas-fir with (left) and without (right) SNC impact (Photo credit: Swiss Needle Cast Cooperative http://sncc.forestry.oregonstate.edu/ and Sky Lan)

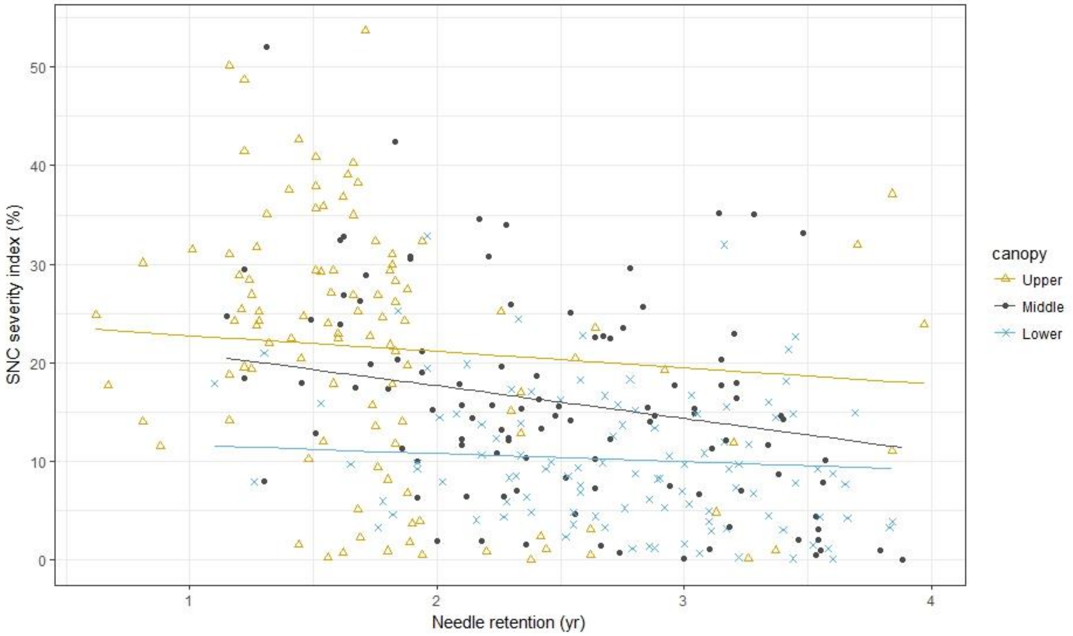

Figure 5.2. Estimated relationships between mean Swiss needle cast disease severity index and needle retention at three canopy levels in young Douglas-fir plantations in western Oregon southwestern Washington, 2013-2015. Regression lines represent samples from the upper, middle, and lower canopy from top to bottom respectively.

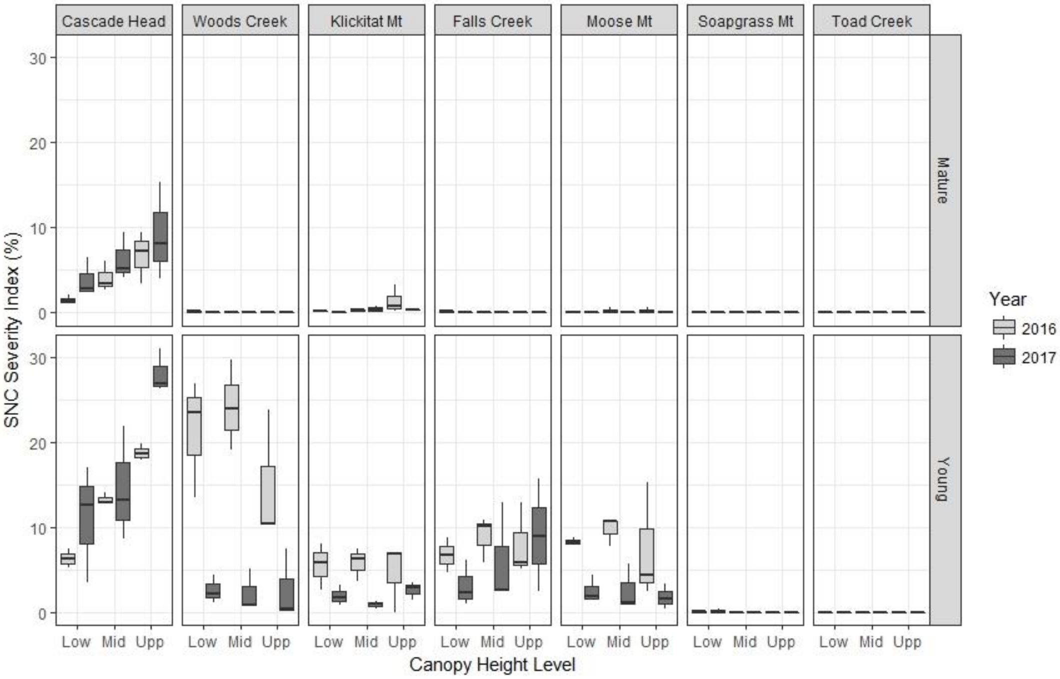

Figure 5.3. SNC severity index at three canopy height levels among 7 sites in western Oregon in 2016-2017. Only 2-year-old needles were used for SNC severity index. The error bar shows the variation from mean.

Figure 5.4. Mature Douglas-fir with (left) and without (right) SNC impact. The left photo was taken in Tillimook in 2018, where is considered as the most SNC severe infection region in Pacific Northwest (Photo credit: Dave Shaw). Trees in the left photo are suppressed by SNC with the low needle retention on twigs, and the sparse, epicormics branches. The right photo was taken in Falls Creek by author in 2015, located in the west slope of Cascade Mountains in Oregon

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APPENDIX: TREE CLIMBING METHODS AND SAFETY PRIORITY

Tree climbing is a fun but also a dangerous activity. NEVER go out for a tree climbing alone. In this dissertation, we used ladders and personal lanyard to “free-climb” the plantation trees, and used the rope systems to climb mature and old-growth trees.

In the fourth chapter of this dissertation, all the tree climbers involved were well-trained and experienced. All gear and ropes used are new and certified and <5 years-old. In addition, although the climbers are professional, we had a pre-season safety and rescue training to make sure all climbers are familiar with each other as well as the different techniques for descending with an injured person.

Trees were rigged by crossbow and modified arrows. We shoot a fishing line for the middle crown branches. To make sure that ropes are on safe branches, we use binoculars to see the pathway and the strength of anchor branches. The fishing line is used to pull a parachute cord through, and then we tie the rope to the cord and pull the rope though. We ascend the rope, rather than climbing the tree until reaching the anchor. Then, a climber moves the rope to the highest location in the tree possible and placed an anchor consisting of a wire rope and pulley. After descending the tree, the rope is pulled down with parachute cord tied to one end and the cord is left in place and the tree can be re-climbed quickly.

To access the tall mature/old-growth Douglas-fir, Single Rope Technique (SRT) is used for vertical movement. Climbing efficiency is the main reason to use SRT. We always anchor the end of the climbing rope on the ground by friction knots or clove hitch. Most of time the ground anchor is the adjacent living tree instead on anchor the sample tree itself. Dynamic Double Rope Technique (DdRT) is used for moving in canopy, especially for branch walking and short ascending/descending. Personal lanyard or working ropes is require for DdRT. Depends on the preference of different climbers, the DdRT systems are various.

Communication is required at the study site before ascending trees, including the climbing and sampling plans, possible ground supports, and the rescue options just in case. When we ascend trees, we always have at least two life supports attached to the harness in case one fails. Helmet is required for head protection. Eyes protection is strongly recommended. All things carried by the climber, including gear, sensors, cables, or personal stuffs (i.e. camera and water bottle), will be attached by a lanyard to the harness to avoid dropping.

Because gear and ropes are important for the life support, we examine all gear and rope EVERY SINGLE TIME before the trip, ensuring no damage and cracks. We retire those ropes and gear once we feel uncomfortable to use, even they look like new. Although all gear is retired after five years no matter the condition. After a field trip, cleaning is needed to remove dust and moss from gear and rope. If we climb in rainy days, all muddy paraphernalia will be washed by water and softy brush, and then dried.

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