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Associations between Swiss Needle Cast Severity and Foliar Nutrients in Young-Growth Douglas-Fir in Coastal Western Oregon and Southwest Washington, USA

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Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii*) caused by *Nothophaeocryptopus gaeumannii*, an ascomycete fungus (Mycosphaerellaceae) that causes tree growth reductions in the Pacific Northwest. The epidemiology of the fungus is generally well known, but the relations between disease expression and foliar nutrition are unclear. In this study, we used data from the Swiss Needle Cast Cooperative research and monitoring plot network in western Oregon and SW Washington to assess associations between SNC severity, carbon, and nine foliage nutrients (nitrogen, Na, K, P, Ca, Mg, Mn, Al, S). Foliage samples were collected from midcrown of selected Douglas-firs from each plot. SNC severity was determined on 2-year-old needles by multiplying disease incidence and fungal reproductive (pseudothecia) density. Disease severity and nutrient relations were determined using linear mixed models. SNC severity showed statistically significant positive trends with concentrations of carbon, nitrogen, Na, K, and S, no relation with concentrations of Ca, Mg, or Al, and slightly negative trends that were not significant for P and Mn. This is the first such analysis of associations between a conifer foliage disease and foliage nutrients across a landscape; subsequently, there is little published literature on how or why these nutrients may interact with disease.

Keywords: conifer foliage diseases, forest disease, forest nutrition

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). The disease causes growth losses in Douglas-fir plantations across coastal Oregon and Washington (Shaw et al. 2011). Swiss needle cast 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 hectares of forest with visible disease symptoms in coastal Oregon, which increased to 238,705 hectares in 2015 (Ritóková et al. 2016). Although mortality from SNC is rare, growth loss of wood volume was estimated at ~23 percent within the NW Coast Range portion of the epidemic area, whereas some plantations experienced up to 52 percent growth losses (Maguire et al. 2002). Management of Douglas-fir stands infected with SNC requires a nuanced approach because impacts vary with geographical setting, climate, and other plantation attributes. Foliage nutrition is a plantation scale attribute that may influence foliage disease, but research on this topic is quite limited.

Nothophaeocryptopus gaeumannii is a widespread, endophytic fungus that plugs the stomates with fungal reproductive structures (pseudothecia). Needles with 50 percent or more plugged stomates will likely die and fall ("cast") from the tree (Hansen et al. 2000). 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.

Nitrogen and Ca in the soil and foliage are hypothesized to play an important role in SNC disease severity (El-Hajj et al. 2004, Perakis et al. 2006, Mulvey et al. 2013). Perakis et al. (2006) showed

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a correlation between increased soil nitrogen and decreased soil Ca following the pattern of increasing SNC severity from 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 El-Hajj et al.'s (2004) study, 10-year-old trees were fertilized, and the authors found a positive association between the concentration of nitrogen in conifer needles and the concentration in the pseudothecia of the fungus, but there was only a weak relation between carbohydrates in pseudothecia and infected needles.

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

There is a general lack of information on foliage disease and foliage nutrition in forestry. This study investigated the associations between SNC severity and common foliar nutrients in a network of research plots established by the Swiss Needle Cast Cooperative (SNCC) (http://sncc.forestry.oregonstate.edu/) in coastal Oregon and southwest Washington, as a first step in assessing the relation between nutrients and SNC on the landscape. The plot network represents a range of disease severity and therefore allows us to ask: Are foliar nutrients in Douglas-fir associated with disease severity caused by *N. gaeumannii*? Based on the results of previous studies (El-Hajj et al. 2004, Perakis et al. 2006, Mulvey et al. 2013), we hypothesized that higher nitrogen and lower Ca in the needle are associated with SNC severity. Associations were also examined between SNC severity and concentrations of other elements in our study, including Na, K, S, P, Mg, Mn, and Al. Carbon was included because it is related to foliar nitrogen and site productivity.

Methods

The study was conducted in Douglas-fir plantations, located within 50 km (35 miles) of the Pacific Ocean, from the California border to southwest Washington (Figure 1). Each plot consisted of a 0.08-hectare (0.2-acre) area of uniform-age trees 10–30 years old. The targeted composition was a minimum of 80 percent Douglas-fir and 740–1,000 trees per hectare (300–400 trees per acre). All plots were established plantations that had not been precommercially thinned or fertilized 5 years prior to establishment. The research plots are located across diverse elevation, latitude, and rain shadow, and so precipitation and temperature vary. Annual precipitation ranges from 1,200 to 4,800 mm, and occurs primarily from October to May. The mean annual temperature ranges from 13 to 18°C, and the elevation of the research plots ranges from 40 to 800 m.

A midcrown branch was destructively sampled on the south side of five to 10 of the largest trees by diameter at breast height in each plot and was used for evaluation of SNC severity and foliar nutrients. Disease severity (Manter et al. 2005) was assessed using 50 individual needles from the 2-year-old needle cohort from each tree sample branch. Needles facing underside up were taped on an index card and stored at –20°C. Each needle was closely inspected for stomatal occlusion of pseudothecia (presence or absence). The percentage of 50 needles with psuedothecia was noted as incidence. On each index card, the first 10 needles with pseudothecia present were then examined with a microscope to determine the number of occluded stomates in three regions (base, mid, and tip) of each needle. The proportion of pseudothecial occlusion for these 10 needles was noted as pseudothecia density. The SNC severity index was calculated as the incidence multiplied by the pseudothecia density (in percentage) (Mulvey et al. 2013), and averaged for all five to 10 trees in each plot.

The concentrations of carbon and nine nutrients (nitrogen, Na, K, S, Ca, P, Mg, Mn, Al) were measured in 1-year-old needles from the midcrown samples. One-year old needles were used because that is the leaf condition that the fungus would find as it colonizes foliage in the first year. These nutrients were chosen because some of their roles are in tree growth (N and P), cell wall stability, solute transport across the cell wall, or general disease resistance (Na, K, S, Ca, Mg, Mn, and Al) (Datnoff et al. 2007). After transporting foliage samples to the lab, approximately 200 one-year-old needles were randomly selected from the midcrown samples of five to 10 trees from each plot and dried for 48 h in a drying oven at 40°C. The dried samples were then ground with a ball grinder and stored in combustion vials to avoid contamination.

The measurement of nutrient concentration was based on dry needle weight. Total nitrogen and carbon were determined on dried and ground foliar material using dry combustion on a Thermo FlashEA 1112 (Thermo Fisher Scientific). Foliar samples were digested using 30 percent H_2O_2 and a 1:10 nitric acid–hydrochloric acid (HNO_3–HCl) acid digestion in conjunction with external heating (EPA method 3050; Benton and Wolf, 1997). Digests and extracts were analyzed for Na, K, S, Ca, P, Mg, Mn, and Al with inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Thermo Scientific ICP-OES 61E.

Mixed linear models were used to test the association of nutrients with disease severity by using R (v. 3.4.3, R Core Team 2017) packages dplyr (Wickham et al. 2017), ggplot2 (Wickham 2009), and nlme (Pinheiro et al. 2017). Separate models were run for each nutrient variable. Nutrient concentration was treated as

Management and Policy Implications

Foliage diseases are thought to be influenced by foliage nutritional status. However, there is very little research on the topic. In Douglas-fir forest plantations of coastal Oregon and Washington, Swiss Needle Cast (SNC) has been at epidemic levels since 1996. It has been hypothesized that the severity of SNC is related to high foliage nitrogen and low foliage calcium, therefore we do not recommend fertilization of Douglas-fir plantations with N. Our new research shows that there is a positive association between N, Na, K, and S in foliage and disease severity across the Coast Range of western Oregon and SW Washington, while Ca, Mg, Al, Mn, and P were not significantly associated. This paper does not describe cause and effect, but managers should be aware that high N sites are at higher risk for disease, while the implications of the positive association with Na, K, and S are more difficult to interpret. We feel that more research is needed to better understand the relationship between foliage nutrition and foliage diseases because nutrient inputs are commonly managed by foresters. Fertilization activities in plantations should include an assessment of how these nutrient additions might predispose trees to foliage diseases.

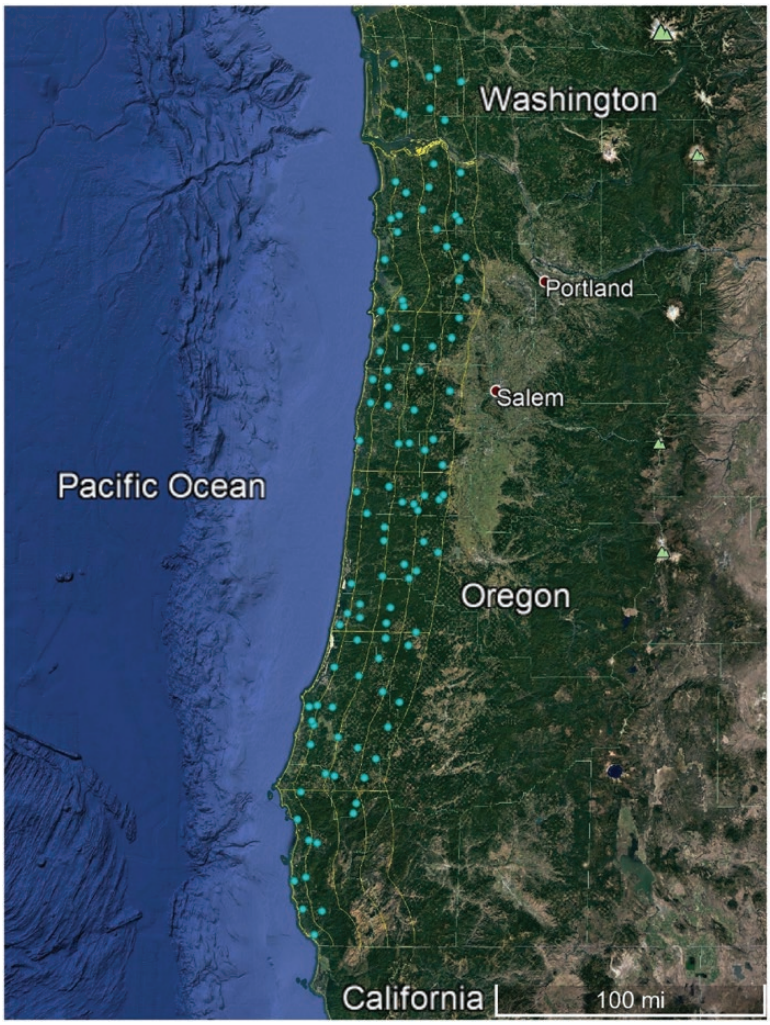

Figure 1. Distribution of 106 research plots established in 2013–15 by the Swiss Needle Cast Cooperative (SNCC) in western Oregon and southwestern Washington (Ritóková et al. 2017). The yellow lines show the geographical grids, and the blue dots represent the research plots. (Photo credit: Google Earth.)

Table 1. Associations between nutrients and SNC disease severity index in 106 plots in coastal Oregon and southwestern Washington, 2013–15.

Nutrient	*F*-value	*P*-value	Estimate of intercept	Estimate of slope
N	20.4	**<.001**	–6.34 (5.55)	14.94 (3.31)
C	25.6	**<.001**	–232.69 (49.18)	5.00 (0.99)
Na	44.0	**<.001**	12.12 (2.17)	0.03 (0.01)
K	8.7	**.004**	–2.01 (6.75)	0.00 (0.00)
S	20.8	**<.001**	–15.76 (7.40)	0.04 (0.01)
Ca	0.0	.84	16.50 (3.84)	–0.00 (0.00)
P	2.3	.13	22.67 (5.08)	–0.01 (0.00)
Mg	0.8	.36	21.41 (3.48)	–0.01 (0.01)
Mn	5.7	**.02**	20.15 (2.83)	–0.01 (0.00)
Al	2.5	.12	19.83 (3.59)	–0.03 (0.02)

Note: Statistical number (*F*-value), significance (*P*-value), and the estimates of intercept and slope were calculated from linear mixed model. Standard errors are shown in parentheses followed by the estimates. Bold value indicates significant of *P*-value ($P < .05$).

the fixed effect, and plots were treated as random effects, which assumed all 106 plots were independent.

Results and Discussion

Concentrations of nitrogen, carbon, Na, K, and S were significantly positively associated with SNC severity index on 2-year-old needles, and concentrations of Ca, P, Mg, Mn, and Al did not show a statistically significant association with mean SNC severity index (Table 1, Figure 2). The research examining relations between foliage diseases and foliar nutrients in conifers is lacking, and much of the information regards response to fertilization (Datnoff et al. 2007) (Table 2). We studied unfertilized plantations across a geographic and climate gradient and found that the higher foliar nitrogen was positively associated with the SNC severity index.

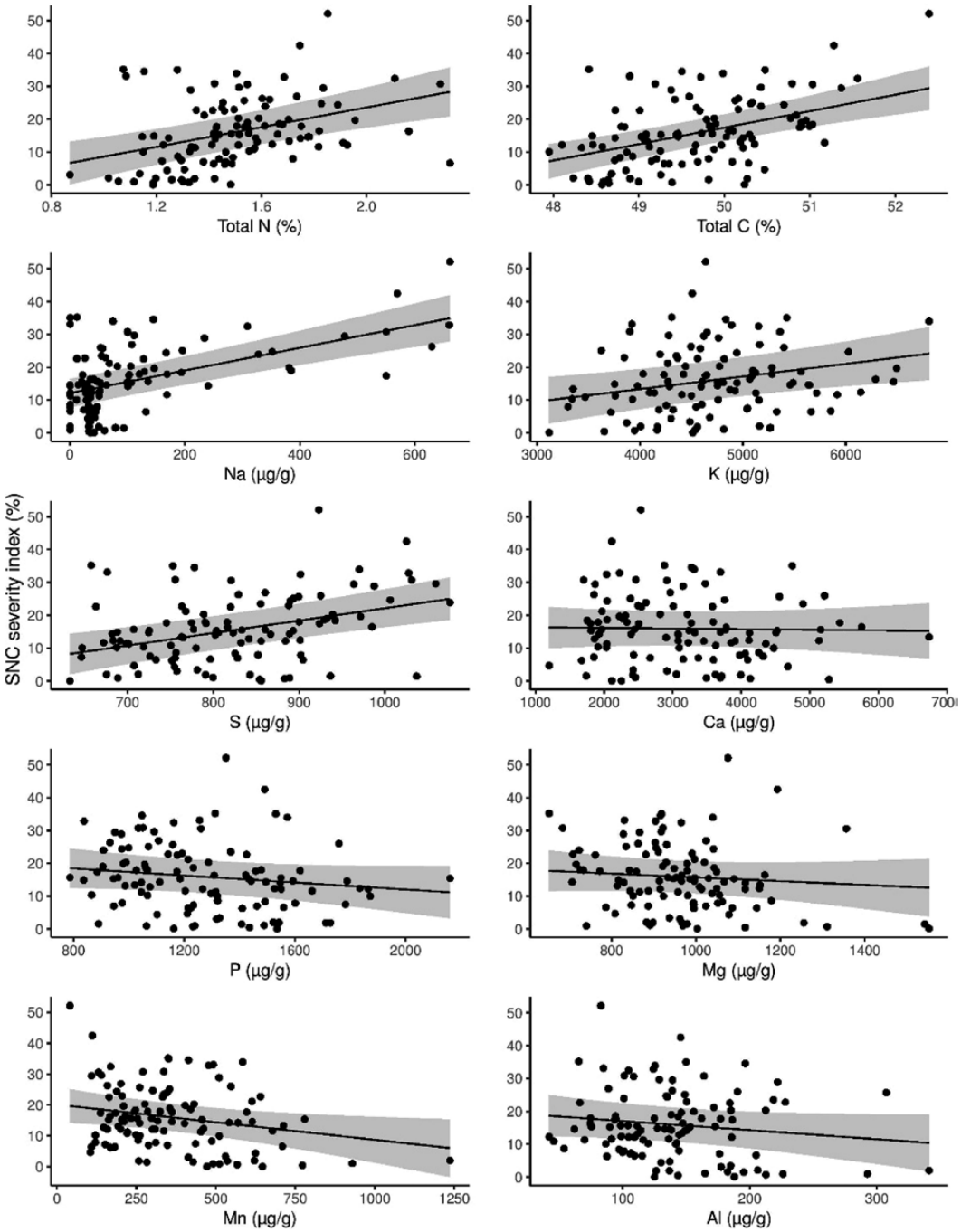

Figure 2. Estimated relations between mean SNC severity index and foliage nutrients at midcrown level in young Douglas-fir plantations in western Oregon southwestern Washington, 2013–15. Shaded areas indicate 95 percent confidence intervals. SNC severity index (percent) is calculated as the incidence (percentage of 50 needles with psuedothecia) multiplied by the pseudothecia density (proportion of pseudothecial occlusion), and represented as a mean of all 5–10 trees in the same plot. Significant positive relations were found for nitrogen, carbon, Na, K, and S ($P < .01$).

The linkage between foliar nitrogen 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) used isotopes to find that a high percentage of nitrogen in fungal pseudothecia was from the host needles. Perakis et al. (2006) suggested that higher soil nitrogen and lower soil Ca were associated with severe SNC, and we found higher foliar nitrogen 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.

Total nitrogen and total carbon concentrations in foliage were positively associated with SNC severity. Foliar carbon concentration is commonly related to foliar nitrogen concentration, as carbohydrates are products of photosynthesis. However, Saffell et al. (2014) found that foliar concentrations of non-structural carbohydrates (NSC) were unrelated to SNC severity.

Table 2. Summary of literature on relations 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 Foliar NSC retains high in infected needles	Saffell et al. (2014)	Positive
K	Pinus needle shedding, K decreases in shedding needle *Pinus strobu* rust (*Peridermium* spp.), K decreases in disease	Bruning 1965, cited in Prabhu et al. (2007a) Hutchinson 1935, cited in Prabhu et al. (2007a)	Positive
Na	Conifer foliage disease relations unknown		Positive
Ca	No effect in fertilization and SNC Conifer foliage disease relations unknown	Mulvey et al. (2013)	None
P	Fusiform rust on pine, P increases in disease No response to fertilization and SNC	Prabhu et al. (2007b) Mulvey et al. (2013)	None
Mg	Included in custom fertilizer blends and no response Conifer foliage disease relations unknown	Mulvey et al. (2013)	None
Mn	Included in custom fertilizer blends and no response Conifer foliage disease relations unknown	Mulvey et al. (2013)	None
Al	Conifer foliage disease relations unknown		None
S	S related to *Dothistroma* severity Conifer foliage disease relations unknown	Lambert (1986), S deficiency related to increased *Dothistroma*	Positive

One possible explanation for the positive relation between nitrogen concentration in 1-year-old needles and SNC severity could be that severe SNC infection on 2-year-old needles causes retranslocation of nitrogen to 1-year-needles. Nitrogen in plants is very mobile and usually concentrated in active tissues (Pallardy 2008). Typically, nitrogen is retranslocated to woody tissues in perennial species (Taiz and Zeiger 2010), but 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 nitrogen to 1-year-old needles as 2-year-old needles die and fall off. Something similar may also be occurring with other highly mobile nutrients (Kimmins 1987), such as Na, K, and S, which also showed associations with disease.

The nutrient, Na, K, and S, may influence the ability of the fungus to access nutrients within the cell. Stone et al. (2008) have shown that *N. gaeumannii* does not penetrate host cell walls but that hypha are appressed to the cell wall of palisade parenchyma and the spongy mesophyll. Sodium and K are known to influence solute transport across the cell wall, and S may increase the availability of other nutrients and plant resistance to disease (Datnoff et al. 2007). In addition, these elements are high in seawater and in areas affected by sea salt deposition (Vet et al. 2014), so the correlation of these compounds with SNC severity could be a result of collinear relations such as soil nitrogen and climatic factors not analyzed here.

Fertilization and management of nutrients to maximize tree growth are fundamental aspects of forestry. Our assessment of young forest plantation foliage nutrients and their relation to foliage disease is important because it can influence management decisions regarding whether to fertilize or not (Mulvey et al. 2013). Mulvey et al. (2013) investigated fertilization response with a variety of applications that included nitrogen, two forms of Ca, P, and custom fertilizer blends (with Mg and Mn), and did not see a response in SNC severity with any treatment, although management recommendations for Douglas-fir forests impacted by SNC have discouraged the use of nitrogen fertilizer based partially on financial merits.

The scope of inference for this study was limited to Douglas-fir plantations in Oregon and SW Washington coastal regions. Although there are associations between foliar nutrients (nitrogen, Na, K, S) and disease severity, we did not show cause--effect, which would require experimental treatments. Explanatory relations between foliar nutrients and *N. gaeumannii* are still unknown, but this study narrowed down the potential nutrients related to SNC severity for further consideration of *N. gaeumannii* infection ecology. It is important to consider that foliar fungi could be influenced by foliar nutrients.

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