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Fertilization impacts on Swiss needle cast disease severity in western Oregon

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ABSTRACT

The influence of fertilization on disease severity is unknown in most forest pathosystems. Fertilization treatments were randomly applied to 0.01 ha plots centered on individual dominant or co-dominant Douglas-fir trees in ten Douglas-fir stands from coastal Oregon to the foothills of the Oregon Cascade Range, USA. This region is affected by Swiss needle cast, caused by the fungal pathogen *Phaeocryptopus gaeumannii*. Selected stands represented a range of Swiss needle cast disease severity, and 10 replications of each fertilization treatment were applied in each stand. The six treatments included nitrogen (urea), calcium as lime (calcium carbonate), calcium as calcium chloride, phosphorus (monosodium phosphate), a site-specific blend (Kinsey) and an unfertilized control. Fertilization took place from February–April 2007, and single branches were collected from treated trees for disease severity assessment of foliage in May 2010. Disease severity of 1- and 2-year-old needles was evaluated by counting the frequency of infected needles and the density of *P. gaeumannii* fruiting bodies (pseudothecia) on a random subset of needles from each tree and needle age class. Fertilization treatment effects on infection index (mean fruiting body density) were tested by mixed-effects models that accounted for site as a blocking factor. Treatment effects on infection index at each of the study locations were also tested by ten separate ANOVAs. Across and within sites, fertilization treatment did not significantly affect infection index of 1- or 2-year old needles ($p > 0.05$). Small differences in mean fruiting body density ($\leqslant$3%) between fertilization and control treatments across sites were not statistically significant, nor are they believed to be biologically or economically significant. Decisions regarding fertilization should be based on site-specific attributes, such as soil chemistry. There is no evidence that fertilization directly ameliorates or exacerbates Swiss needle cast severity in western Oregon.

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1. Introduction

Swiss needle cast (SNC) is a foliage disease of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) caused by the ascomycete fungus *Phaeocryptopus gaeumannii* (Rhode) Petrak. Fungal fruiting bodies (pseudothecia) occlude needle stomata, which impairs gas exchange and leads to needle carbon starvation and abscission at high infection levels (Manter et al., 2000, 2003). Disease symptoms include premature needle shed, chlorotic needles and tree crowns, and reduced height and diameter growth (Hansen et al., 2000; Maguire et al., 2002). Although the fungus is native and endemic within the range of Douglas-fir in western North America and was long considered innocuous in this region, a foliage epidemic affecting hundreds of thousands of acres has emerged in the Coast Range of Oregon and Washington since the 1990s (Shaw et al., 2011). Many questions have arisen about the potential of various silvicultural treatments to ameliorate the disease (Filip et al., 2000).

Several climate factors are correlated with moderate and severe disease. These include abundant leaf wetness from fog and precipitation during the sporulation period (especially May–July), and mild temperatures during the winter, which are believed to allow for more rapid needle colonization (Rosso and Hansen, 2003; Manter et al., 2005; Stone et al., 2008a). Climate conditions conducive to disease development are generally found at low elevations within 20–30 miles (30–50 km) of the coast, on sites that were historically dominated by Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophylla*) and red alder (*Alnus rubra*) (Hansen et al., 2000). In western Oregon (USA), distance-from-coast and elevation consistently emerge as strong predictive variables in disease severity distribution models (Hansen et al., 2000; Rosso and Hansen, 2003; Manter et al., 2005; Zhao et al., 2011). While distance-from-coast and elevation are not mechanistic explanatory variables, they act as surrogates for climate and site variables that change along these gradients and have a significant impact on disease development. Soil N concentration is negatively correlated with distance-from-coast (Perakis et al., 2005), and there has been

Abbreviations: SNC, Swiss needle cast.

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speculation about possible predisposing effects of N levels and nutrient imbalance on disease development and severity (El-Hajj et al., 2004). As a result, managers are interested in the potential of fertilization regimes that may ameliorate or offset growth losses from SNC, and currently avoid regimes that may exacerbate the disease and growth loss.

In several agricultural pathosystems, N, Ca, P and other fertilizer treatments have been shown to directly impact disease severity, but the direction of impact is often dependent on the pathosystem, the specific application materials (e.g., ammonium, nitrate or nitrite), the timing of application, and other factors (Engelhard, 1989; Datnoff et al., 2007). Nutrient manipulation may reduce disease by bolstering plant resistance or tolerance through enhancing the production of compounds and signaling molecules associated with chemical or physical defense, for example, soluble and cell wall-bound phenolics, monoterpenes, lignin and 'second messengers' (Bonello et al., 1993). Conversely, nutrient manipulation may increase disease by improving pathogen access to nutriment through its host or by negatively impacting the production or distribution of host defense compounds in plant tissue. Sugimoto et al. (2010) demonstrated that soil Ca applications to soybean crops improved resistance to the stem rot pathogen, *Phytophthora sojae*. Accumulation of Ca crystals around the cambium and xylem elements of treated plants indicated that Ca served as a barrier to vascular tissue penetration. Fertilization treatments, such as lime, may also alter soil pH, thereby affecting the availability and uptake of other charged soil nutrients (e.g., P), not only the nutrients directly applied (Haynes, 1982; Punja, 1989). Changes in soil acidity may alter the composition of soil microbial communities, and in some cases, may affect population levels of microorganisms antagonistic to soilborne pathogens, thereby influencing disease levels (Punja, 1989).

Comparatively fewer studies have been conducted on the influence of fertilization or nutrient levels on pathogens of forest trees, at least in part due to the long-lived nature of trees and the complexity of nutrient cycling in forest systems. In addition, observational studies on the relationship between mineral nutrient concentrations and disease severity do not allow causal inferences to be drawn, because nutrient concentrations are confounded with other environmental factors and tree physiological conditions. A field experiment conducted by Blogett et al. (2005) found that N fertilization decreased resistance of red pine (*Pinus resinosa*) to the fungal shoot blight and canker pathogen, *Diplodia pinea* (syn. *Sphaeropsis sapinea*). Although it is generally believed that stressed trees are predisposed to disease in this pathosystem, and fertilization is often recommended to increase tree vigor, significantly lower lignin concentrations were detected in fertilized trees compared to controls. Wallis et al. (2011) investigated the influence of N fertilization of Austrian pine (*Pinus nigra*) inoculated with *D. pinea*, and found that lesion size was negatively correlated with levels of soluble phenolics and monoterpenes, and that significantly higher levels of these compounds were present at high and low N fertilization levels compared with the intermediate level. An observational study conducted by Stanosz et al. (2004) on the influence of paper mill waste treatment on the *D. pinea*-red pine pathosystem detected significantly higher incidence of infected trees and shoots in treated stands. It was suggested that nutrient imbalance (high N relative to essential micronutrients) may have increased disease severity in treated stands by intensifying the effects of moisture stress, because latent infection can develop into severe infection under drought conditions.

In the *P. gaeumannii*-Douglas-fir pathosystem, nearly all infection occurs in current-year foliage during shoot elongation. *P. gaeumannii* infects through needle stomata, and then colonizes the intercellular region of host needles. Fungal biomass increases in needles as they age and is positively correlated with fruiting body density, resulting in greater density of occluded stomata over time (Stone et al., 2008b). Manter et al. (2003) demonstrated that deleterious effects (reduced carbon assimilation) of fungal infection and colonization were only observed after fungal fruiting bodies developed in needle stomata, and that fungal-mediated reduced carbon uptake is the primary disease mechanism. *P. gaeumannii* can grow within needles without causing symptom development or measureable growth loss as long as the photosynthetic capacity of efficient, young (1- to 2-year-old) needles is not compromised by stomatal occlusion (Hood, 1982). Premature abscission must then be attributable to high levels of successful initial infection, accelerated colonization and fruiting body development under certain environmental conditions and host nutritional states, or both (Manter et al., 2005).

Some have speculated that high levels of N relative to other macro and micronutrients may increase nutrient availability in the apoplast where it can be accessed by *P. gaeumannii* (El-Hajj et al., 2004; Perakis et al., 2005). In the region of the epidemic in the Pacific Northwest (USA), foliar N levels often exceed the established 1.4% threshold for N-limitation in coastal Oregon Douglas-fir (Perakis et al., 2005). El-Hajj et al. (2004) fertilized Douglas-fir trees with two levels of N (urea) in Idaho and documented 2.2- to 3.6-times greater pseudothecia density on 2-year-old needles of treated trees. The small sample size (5 trees/treatment), the intermountain west study location, and the *P. menziesii* ssp. *glauca* seed source suggest that further research and verification is needed before extending these findings to forest plantations in coastal Oregon and Washington. In the north-central Oregon Coast Range, Perakis et al. (2005) measured soil and foliar nutrition in 22 stands across a gradient of SNC. Douglas-fir foliar N levels, which ranged from 0.85% to 1.74%, were positively correlated with soil N levels and negatively correlated with foliage retention. It was suggested that nitrate-leaching of Ca on N-rich sites, combined with low rates of atmospheric Ca deposition relative to tree demands, contributed to Ca depletion and N oversaturation on coastal sites, possibly contributing to low needle retention. It is difficult to interpret the relationship between foliar and soil Ca and N levels and SNC severity in observational studies, as these factors covary with distance-from-coast along with many climatic variables that are known to strongly influence abundance of the causal fungus (Manter et al., 2005; Stone et al., 2008a).

N fertilization (as urea) has been a common management practice for increasing tree growth and yield in the Douglas-fir region of the Pacific Northwest (Bengston, 1979), but some foresters in coastal Oregon and Washington are concerned that, in areas of moderate to severe SNC, N fertilization may worsen disease severity (Filip et al., 2000). Plantations are traditionally fertilized with urea at the time of pre-commercial thinning (8- to 15-years-old) or commercial thinning (20- to 25-years-old), and may be fertilized at regular intervals (e.g., every 5 years) until harvest (Personal communication M. Gourley, Starker Forests, Inc., Corvallis, OR, USA, January 2011). Influenced by theories regarding nutrient imbalance and SNC, some managers are fertilizing with lime to ameliorate growth loss from disease by lowering the ratio of N to other essential nutrients. Ca and alternative or site-specific fertilization treatments can be costly, and their benefits, in terms of tree growth and disease impacts, have not been thoroughly tested in the region of the current SNC epidemic.

The primary objective of this study was to evaluate differences in disease severity between trees treated with specific nutritional amendments across a range of SNC disease severity in western Oregon. This objective was pursued by implementing a controlled fertilization experiment at 16 study locations chosen to represent a range of disease severity (unpublished: Mainwaring et al., 2009; Mainwaring and Maguire, 2010). Treatments included nitrogen (urea), calcium carbonate (lime), calcium chloride, phosphorous

(monosodium phosphate), a site-specific blend (Kinsey) and an unfertilized control. Analysis of growth responses to these fertilization treatments across a range of SNC severity has been reported separately (unpublished: Mainwaring et al., 2009; Mainwaring and Maguire, 2010).

2. Materials and methods

2.1. Study site descriptions

Sites were distributed across a range in elevation, aspect and SNC severity from the Oregon and southwest Washington Coast Ranges to the foothills of the Cascade Range (Table 1; Fig. 1). Target stands were 10–30 years old, contained 740 (495–985) trees per hectare and had not been previously thinned or fertilized for at least 7 years. Sites were broadly distributed through the region of interest and were chosen to avoid prior fertilization. Tree and stand attributes, and mineral soil and foliar chemistry, were assessed in all stands before treatment in fall 2006, and one and three growing seasons after treatment (unpublished: Mainwaring et al., 2009; Mainwaring and Maguire, 2010).

Studied stands ranged from the coastal Sitka spruce (*P. sitchensis*) vegetation zone inland to the western hemlock (*T. heterophylla*) vegetation zone, which spans much of western Oregon and Washington (Franklin and Dyrness, 1973). The Sitka spruce vegetation zone is generally found within a few kilometers of the coast below

Table 1
Name and site information for 10 Douglas-fir fertilization study sites in western Oregon assessed for Swiss needle cast disease severity.

Site	Location	Landowner[a]	Location	Elevation (m)	Distance (km)[b]	Slope (%)	Aspect	Fol. Ret.[c]
GDH	Hemlock	Stimson (green diamond[a])	T3S, R9 W, S8	122	11.6	10	S	1.62 (0.45)
MNS	Menasha-south	Cambell (menasha[a])	T26S, R13 W, S10	61	11.6	30	E	2.66 (0.71)
ODF	Elk city	Oregon dept. forestry	T11S, R10 W, S1	152	17.7	30	N	2.34 (0.63)
HAGR	Grand Ronde	Hampton	T5S, R8 W, S6	366	20.1	35	NE	2.22 (0.67)
MNN	Menasha-north	Cambell (menasha[a])	T26S, R11 W, S6	244	20.9	10	S	2.22 (0.57)
HAK	Knappa	Hampton	T8 N, R7 W, S29	183	28.2	10	N	2.36 (0.72)
STR	Burnt woods	Starker	T11S, R7 W, S30	305	38.6	10	N	2.71 (0.69)
OSU	McDonald forest	Oregon State University	T9S, R5 W, S4	91	59.5	0	–	3.32 (0.51)
GPH	Pleasant hill	Giustina L&T	T19S, R2 W, S24	305	102.2	10	N	3.64 (0.38)
CTC	Sweethome	Cascade timber	T12S, R1E, S28	442	111	15	S	3.38 (0.83)

[a] Landowner at start of study.
[b] Distance is km from the Pacific Ocean.
[c] Mean foliage retention (Fol. Ret.) provided for 2006 (pre-treatment); std. dev. in parentheses.

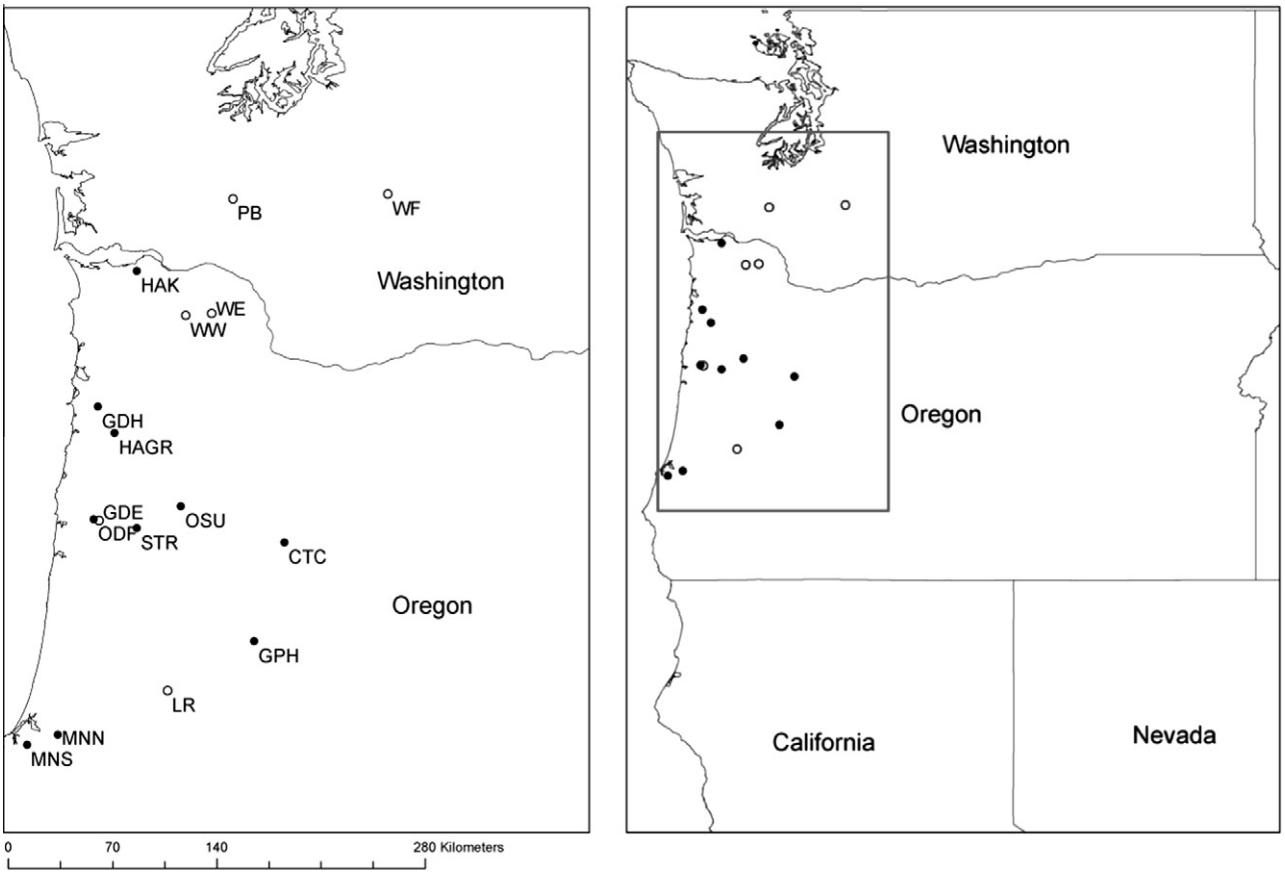

Fig. 1. Douglas-fir fertilization study locations in the Oregon and Washington Coast Ranges and the foothills of the Cascade Range, Pacific Northwest, USA. Black circles represent stands that were sampled for disease severity assessment; white circles represent stands that were not.

150 m in elevation, but extends farther inland along river drainages. This zone tends to have the highest SNC severity, and is characterized by a consistently wet and mild climate, with minimal moisture stress and frequent fog and low cloud in summer. Soils in this zone are deep, rich and fine-textured, and surface soils tend to have high organic and total N content, and low pH (4.5–5.5) and base-cation saturation. Sitka spruce, western hemlock, Douglas-fir, western redcedar (*Thuja plicata*) and red alder (*A. rubra*) dominate this highly productive region (Franklin and Dyrness, 1973).

The more extensive western hemlock zone tends to experience relatively greater temperature and moisture extremes that the Sitka spruce zone, and there is substantial climatic variation associated with the wide range of elevation, latitude, longitude and regional topography. Most precipitation occurs during winter, while summer receives less than ten percent of the annual total. Soils tend to be moderately deep and somewhat acidic, and, in the Coast Ranges, organic matter is high. Dominant tree species include Douglas-fir and climax western hemlock and western redcedar (Franklin and Dyrness, 1973).

2.2. Fertilization regimes and soil collection

Ten of the 16 study locations were selected for foliage collection and disease severity assessment, with preference given to sites that were located in Oregon and that provided a range of SNC disease severity. Treatments included nitrogen (urea), calcium as lime (calcium carbonate), calcium as calcium chloride, phosphorus (monosodium phosphate), a site-specific blend (Kinsey) and an unfertilized control. Of the 10 study sites, nine received the six main fertilization treatments, while one (GPH) received all treatments except the site-specific blend (Table 2). One site (OSU) also received an additional N + P treatment (448 kg ha^{-1} N and 112 kg ha^{-1} P) applied as a blend of urea and monoammonium phosphate. The Kinsey treatment targeted specific base-cation saturation ratios (McLean et al., 1983), and both elements and rates were prescribed based on analysis of soil samples collected beneath treatment trees and pooled within treatments at each site (Oregon State University Central Analytical Laboratory, Corvallis, OR, USA). Two mineral soil core samples were collected (12.5-cm depth with the duff layer eliminated) 1.5 m from opposite sides of each treatment tree and perpendicular to the slope. A third soil sample that included the duff layer was collected from soil beneath trees assigned to the Kinsey treatment. The Kinsey treatment prescribed addition of Cu, S and Ca (as lime) to all sites; N, P and K to all but one of the nine sites (STR); and various combinations of dolomitic lime and other micronutrients (Table 3).

Treatments were ground-applied and randomly assigned to circular 0.01 ha (5.67 m radius) fixed-area plots, with each plot centered on an undamaged dominant or co-dominant measurement tree. Trees were considered damaged if they were leaning or had broken tops, forked tops or bole wounds. Potential plot center trees were selected on a 20-m grid, and grid points were skipped if no

Table 2
Application rates and materials defining fertilization treatments applied to 0.01-ha plots centered on dominant or co-dominant Douglas-fir trees at study sites in western Oregon. Kinsey treatment applications rates displayed in Table 3.

Treatment	Material	Chemical formula	Material appl. rate (kg ha^{-1})	Elemental appl. rate (kg ha^{-1})	Expected outcome
Control	–	–	–	–	–
N	Urea	$(NH_2)_2CO$	493	225	Increased N
Ca	Lime (calcium carbonate)	$CaCO_3$	2915	1020	Increased Ca and soil pH
Ca	Calcium chloride	$CaCl_2$	291	105	Increased Ca w/o pH change
P	Monosodium phosphate	NaH_2PO_4	2240	580	Increased P and soil pH

Table 3
Kinsey fertilization application rates and materials applied to 0.01-ha plots centered on dominant or co-dominant Douglas-fir trees at 9 sites in western Oregon.

	Sites[a]								
	GDH	MNS	ODF	HAGR	MNN	HAK	STR	OSU	CTC
Fertilizer application rates (kg ha^{-1})									
NH_4H_2POH	255	255	255	255	255	255	–	255	255
K_2SO_4	127	–	–	127	–	–	–	433	509
S_8	97	97	92	92	87	81	97	97	107
Borate-46	–	5	10	15	–	7	15	15	15
$ZnSO_4$	20	–	20	20	20	–	20	10	–
$CuSO_4$	20	25	25	10	25	25	20	20	10
$FeSO_4$	–	407	407	331	407	407	–	433	407
$K_2Mg_2(SO_4)_3$	305	407	407	305	330	407	–	–	–
$MnSO_4$	102	–	–	–	–	–	–	–	–
$CaCO_3$	1222	2291	2291	1935	967	1527	2851	2138	4939
$CaMg(CO_3)_2$	3157	1731	2189	2749	3259	2138	2546	–	4226
Elemental application rates (kg ha^{-1})									
N	31	31	31	31	31	31	–	31	31
P	69	69	69	69	69	69	–	69	69
K	113	74	74	113	60	74	–	194	228
S	209	269	269	250	247	255	97	265	279
Ca	464	871	871	735	367	580	1083	812	1877
Mg	444	270	329	391	460	323	331	–	549
Mn	29	–	–	–	–	–	–	–	–
B	–	1	1	2	–	1	2	2	2
Zn	7	–	7	7	7	–	7	4	–
Cu	5	6	6	2	6	6	5	5	2
Fe	–	85	85	70	85	85	–	91	85

[a] Sites ordered by increasing distance from coast.

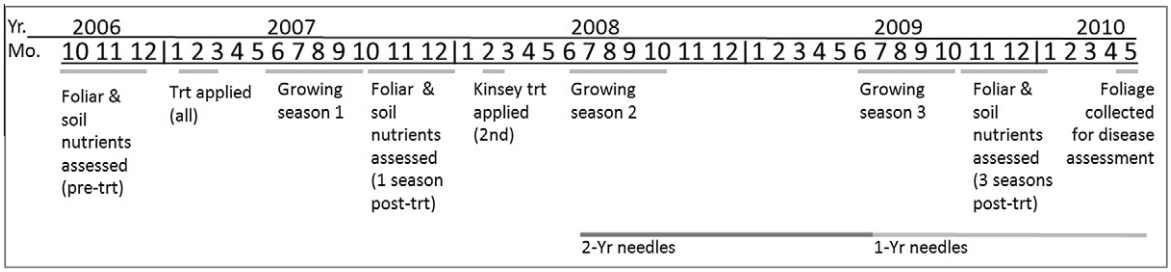

Fig. 2. Timeline of activities required for Douglas-fir the fertilization study, including foliar and soil nutrient analyses, treatment (trt) applications, and disease assessment relative to the three growing seasons.

suitable subject trees were available. At each site, fertilization treatments were replicated ten times, and treatments were applied February–April 2007 (Fig. 2). For the Kinsey treatment, attaining the specified ratio of base cations required fertilizer application across 2 years. Non-lime materials were applied at the start of the experiment with the other fertilization treatments, and lime was applied during the following winter of 2007–2008. Mineral soil samples were collected one and three growing seasons after treatment for assessment of fertilization impacts on soil chemistry.

Materials were chosen to allow for investigation of the comparative impacts of N fertilization at rates commonly used in Douglas-fir plantations in the study region (Peterson and Hazard, 1990; Talbert and Marshall, 2005). Douglas-fir is not operationally fertilized with other nutrients, so their rates were based in part on those applied in other species and regions (e.g., Albaugh et al., 2007), in part on rates applied in agricultural systems (Kinsey and Walters, 2006), and in part on rates expected to test the limiting effects of specific nutrients and/or the indirect effects of changes in soil pH (Havlin et al., 2004). To examine the effects of specific nutrient additions on tree growth and disease severity, treatments were designed to elevate the initial availability of a specific elemental nutrient, with the exception of the Kinsey regime that involved a blend of nutrients. Two Ca treatments were applied to distinguish between the impacts of Ca accompanied by increased soil pH (lime) and the impacts of Ca alone (calcium chloride). Industrial rates of N were used to represent operational plantation scenarios, while agricultural rates were selected for forest fertilization not traditionally practiced in the study region. Fertilization treatments were not intended to elevate soil or foliar nutrient levels in excess of what would be expected under standard plantation management.

2.3. Foliage collection for disease assessment and foliar chemistry

One- and 2-year-old foliage was collected from treatment trees in May 2010 for disease severity assessment (Fig. 2). Tree climbers removed the largest 4-year-old lateral branch from the southernmost branch of the fifth whorl from the top of the each treatment tree (Maguire and Kanaskie, 2002; Maguire et al., 2002). Approximately half of the needles were removed from the primary branch axis of the selected 4-year-old lateral branch, and half were removed from higher order branches growing off of this lateral. One- and 2-year-old needles from each tree were stored separately in labeled polyethylene bags for disease severity assessment. The minimum number of needles for sampling a given age class on a given tree was set at 30, but 25 trees either had no new growth or retained insufficient 1- or 2-year-old needles on the collected branch. At least seven of the 10 trees per treatment had sufficient needles at each site; in total, 1120 samples from 565 trees were assessed for disease severity.

Branches were also collected before treatment (October–December 2006), one growing season after treatment (October–December 2007) and three growing seasons after treatment (October–December 2009) for foliar chemistry assessment (Fig. 2). One-year-old needles from trees in the same treatment group at each site were pooled and mixed, and approximately 1 g of dried foliage per sample was submitted for analysis (Oregon State University Central Analytical Laboratory, Corvallis, OR, USA). Foliage was collected for nutrient analysis in autumn, when nutrient levels are believed to stabilize after the growing season. One-year-old needles collected in October–December 2009 for nutrient analysis were from the same cohort as the 1-year-old needles collected in May 2010 for disease severity assessment; i.e., both of these collections were made prior to the 2010 growing season. Foliage collection for disease assessment we delayed until spring, when a greater proportion of *P. gaeumannii* fruiting bodies have matured and are visible. Foliar nutrients were assessed as percent or $\mu g\ g^{-1}$ dry weight.

2.4. Foliage processing and disease severity assessment

Foliage was processed for disease severity using methods developed by Hansen et al. (2000), with minor modifications. For each tree and needle class, 50 needles were randomly selected and attached to a 3×5 index card with double-sided tape, such that abaxial needle surfaces faced upward. Stereo dissecting microscopes ($40\times$ magnification) were used to determine the proportion of needles on which *P. gaeumannii* fruiting bodies (pseudothecia) were present (infection incidence).

The first ten needles on which the fungus was present were marked from 1 to 10, and pseudothecia density on each of these needles was examined at approximately $210\times$ magnification with digital microscopes (Dino-Lite Pro AM413ZT- Polarizing) using compatible DinoCapture 2.0 software (AnMo Electronics Co., Torrance, CA). Counts of the proportion of stomata occluded by pseudothecia (pseudothecia density) were conducted at three distinct locations along the needle, with the needle divided into three sections of equal length (base, middle, and tip) given the total length of the needle. In each needle-third, a random starting point for the count was selected, and 100 total stomata were examined (50 on either side of the needle midrib). Stomata were examined consecutively, moving perpendicular to the midrib, and the number of stomata occluded by pseudothecia (out of 100 stomata examined) in each needle-third was recorded.

Damage or other species of foliar fungi (e.g., *Stomiopeltis*, *Rasutoria psuedotsugae*, etc.) occasionally obstructed the visibility of needle stomata. When this occurred, damaged regions were bypassed until the count could be resumed. Pseudothecia density was then averaged by needle, and across needles from the same tree and cohort, to provide an estimate of mean pseudothecia density for each tree/needle-cohort sample. Infection index, the

product of mean pseudothecia density and infection incidence (Manter et al., 2005), was then calculated for each tree/needle-cohort sample to provide an overall estimate of mean pseudothecia density that accounted for the fact that not all needles were infected.

2.5. Statistical analyses

One-way ANOVA was used to evaluate treatment effects on soil and foliar chemistry across sites (Insightful Corp., S-PLUS, 2007). Needle age classes were analyzed separately for all comparisons related to disease severity. Mixed effects analysis was used to assess fertilization treatment effect on infection index (average pseudothecia density) after accounting for site as a random block effect, and also allowed for *t*-test comparisons between infection indices of individual treatments and the control (Insightful Corp., S-PLUS, 2007). Beta-coefficients from mixed effects analysis were used to rank treatments across sites. One-way ANOVA was used to assess within site treatment differences in infection index, and beta-coefficients from these tests were used to rank treatments within sites (Insightful Corp., S-PLUS, 2007). Simple linear regression models evaluated the relationship at the site level between infection index in May 2009 and foliar nutrients at two different times: October–December 2006 (before treatment), and October–December 2009 (3 years after treatment) (Insightful Corp., S-PLUS, 2007). Site-level values were obtained by pooling and averaging infection indices for all sample trees at each site, and pooling and averaging nutrient levels across all treatments at each site.

Standard methods were used to verify that data met the assumptions of statistical tests. Graphical representations were used to assess distribution shape and variance. Sites and needle age classes with minimal disease had low variance in infection index and positive skew compared to sites with moderate or severe disease. Inclusion of data from low-severity sites did not alter statistical conclusions; therefore, these data were retained in the mixed effects analyses. Statistical tests were performed on untransformed data. The arcsine square root transformation was considered, but use of the transformation did not alter statistical conclusions nor did it uniformly improve distribution of residuals across the dataset. Treatments were randomly applied, allowing for causal inferences regarding treatment effect. The scope of inference was the population of young Douglas-fir plantations that have not been previously fertilized, in most cases because sites were judged to have a lower probability of growth response; however, as indicated below by both initial foliar chemistry and growth responses to N, the scope of inference is probably wider because identifying the population of responding Douglas-fir stands has traditionally been very difficult (e.g. Peterson and Hazard, 1990).

3. Results

3.1. Soil chemistry

Soil nutrient levels and pH were correlated with distance-from-coast, consistent with the trends observed by Perakis et al. (2005). In general, soil pH, Ca, K, Mg and P increased with distance-from-coast, while C, N and Na decreased (Table 4).

Most fertilization treatments caused expected changes in soil pH, P, and Ca (Table 5). The N treatment did not significantly increase soil N, and increases in soil N for the control treatments at several sites complicated interpretation. The N treatment lowered soil pH at all but one site (HAK) and reduced Ca at most sites (data not shown), but these changes were not statistically significant. Lime and Kinsey treatments significantly increased soil Ca (Table 5), with relatively larger increases on sites closer to the coast with lower initial levels of soil Ca (data not shown). The

Table 4
Mean initial soil nutrient and pH levels (before treatment) in the Douglas-fir fertilization experiment by site. Standard deviation in parentheses.

Site[a]	pH	C (%)	Ca (μg g^{-1})	K (μg g^{-1})	Mg (μg g^{-1})	N (%)	Na (μg g^{-1})	P (μg g^{-1})
GDH	4.7 (0.1)	11.4 (0.8)	107 (16)	126 (13)	64 (10)	0.59 (0.03)	54 (12)	0.3 (0.3)
MNS	5.2 (0.1)	8.8 (1.0)	529 (72)	276 (23)	180 (13)	0.46 (0.05)	56 (2)	1.9 (0.1)
ODF	4.9 (0.1)	9.3 (0.8)	343 (108)	265 (34)	175 (43)	0.49 (0.05)	46 (2)	2.5 (1.4)
HAGR	5.0 (0.1)	9.3 (1.4)	550 (95)	258 (40)	230 (36)	0.49 (0.08)	63 (13)	1.1 (0.3)
MNN	4.6 (0.4)	12.4 (1.9)	155 (36)	231 (14)	65 (14)	0.80 (0.10)	56 (13)	2.0 (0.4)
HAK	5.1 (0.1)	11.2 (1.0)	260 (31)	155 (13)	84 (11)	0.55 (0.04)	65 (17)	5.5 (1.1)
STR	5.4 (0.1)	5.0 (0.3)	1216 (130)	399 (20)	344 (37)	0.28 (0.01)	42 (1)	15.9 (2.0)
OSU	6.3 (0.2)	3.1 (0.2)	2651 (354)	445 (55)	300 (42)	0.21 (0.01)	33 (6)	22.0 (5.2)
GPH	5.9 (0.1)	4.2 (0.3)	1782 (242)	347 (37)	264 (34)	0.19 (0.01)	27 (1)	13.8 (4.0)
CTC	5.4 (0.1)	6.6 (0.3)	3400 (288)	523 (46)	660 (29)	0.32 (0.01)	41 (1)	0.9 (0.3)

[a] Sites ordered by increasing distance from coast.

Table 5
Average 3-year change (final–initial) and average proportional 3-year change (final – initial)/initial) in soil chemistry by treatment (trt) in the Douglas-fir fertilization experiment. Standard deviation in parentheses. Bold indicates nutrient changes that were targeted by trts.

Trt	pH	Ca (μg g^{-1})		N (%)		P (μg g^{-1})	
	Change	Change	Proportional	Change	Proportional change	Change	Proportional change
Control	−0.09 (0.31)	−96 (177)	0.03 (0.27)	0.04 (0.05)	0.07 (0.10)	2 (2)	1.64 (2.48)
N	−0.04 (0.07)	−134 (193)	−0.10 (0.13)	**0.01 (0.06)**	**0.03 (0.10)**	2 (3)	2.20 (3.92)
Lime	**0.25 (0.08)****	**456 (228)****	**1.63 (2.01)****	0.04 (0.05)	0.07 (0.11)	2 (4)	1.79 (2.09)
$CaCl_2$	0.01 (0.08)	**−35 (337)**	**0.25 (0.45)**	0.04 (0.05)	0.08 (0.11)	2 (1)	1.04 (1.41)
P	**0.27 (0.10)****	−78 (162)	0.06 (0.35)	0.02 (0.03)	0.03 (0.07)	**48 (31)****	**28.79 (39.29)****
Kinsey	**0.13 (0.09)****	**292 (181)****	**1.26 (1.70)****	0.02 (0.04)	0.02 (0.08)	4 (2)	3.16 (3.47)
N + P[a]	−0.08	−10	0	**0**	**0**	**20**	**0.93**

[a] Treatment only applied at site OSU.
*Significant change in nutrient level relative to the control treatment ($p \leqslant 0.05$).
** Significant change in nutrient level relative to the control treatment ($p \leqslant 0.01$).

Table 6
Mean initial foliar nutrient levels (before treatment) in the Douglas-fir fertilization experiment. Standard deviation in parentheses.

Site[a]	N: Ca	Ca (%)	K (%)	Mg (%)	N (%)	P (%)	B ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
GDH	8.89 (1.14)	0.17 (0.02)	0.63 (0.03)	0.10 (0.00)	1.49 (0.06)	0.12 (0.01)	13 (1)	75 (8)	10 (1)
MNS	4.94 (0.40)	0.29 (0.03)	0.68 (0.05)	0.09 (0.01)	1.44 (0.04)	0.11 (0.01)	15 (1)	62 (4)	12 (1)
ODF	5.09 (0.80)	0.30 (0.05)	0.76 (0.04)	0.15 (0.02)	1.52 (0.05)	0.14 (0.01)	17 (3)	76 (8)	12 (1)
HAGR	5.03 (0.45)	0.32 (0.02)	0.76 (0.05)	0.13 (0.01)	1.60 (0.13)	0.14 (0.01)	15 (2)	73 (4)	12 (1)
MNN	6.90 (0.45)	0.21 (0.02)	0.58 (0.02)	0.09 (0.00)	1.42 (0.08)	0.11 (0.01)	16 (1)	68 (5)	9 (0)
HAK	4.94 (0.63)	0.27 (0.04)	0.69 (0.03)	0.12 (0.00)	1.29 (0.03)	0.14 (0.01)	17 (1)	70 (11)	13 (1)
STR	2.53 (0.19)	0.52 (0.06)	0.78 (0.03)	0.13 (0.00)	1.31 (0.06)	0.17 (0.01)	19 (1)	87 (20)	13 (1)
OSU	2.05 (0.22)	0.64 (0.05)	0.79 (0.05)	0.14 (0.01)	1.30 (0.05)	0.18 (0.01)	26 (2)	115 (14)	10 (1)
GPH	2.45 (0.14)	0.52 (0.02)	0.85 (0.04)	0.13 (0.01)	1.27 (0.03)	0.15 (0.01)	20 (1)	163 (60)	11 (1)
CTC	2.47 (0.15)	0.55 (0.04)	0.77 (0.04)	0.15 (0.01)	1.36 (0.08)	0.15 (0.01)	24 (3)	156 (56)	19 (4)

[a] Sites ordered by distance from coast.

Table 7
Average 1-year and 3-year change (final-initial) and proportional change (final-initial/initial) in foliar nutrient levels by treatment (trt). Standard deviation in parentheses. Bold indicates nutrient changes that were targeted by trts.

Trt	Ca (%)		N (%)		P (%)	
	Change	Proportional change	Change	Proportional change	Change	Proportional change
Year 1 vs. Initial						
Control	−0.07 (0.04)	−0.18 (0.12)	−0.10 (0.16)	−0.07 (0.11)	−0.01 (0.01)	−0.09 (0.08)
N	−0.05 (0.06)	−0.12 (0.13)	**0.14 (0.23)****	**0.11 (0.17)****	−0.03 (0.01)*	−0.19 (0.08)*
Lime	**−0.05 (0.04)**	**−0.15 (0.10)**	−0.11 (0.13)	−0.07 (0.09)	−0.01 (0.01)	−0.10 (0.09)
$CaCl^2$	**−0.04 (0.06)**	**−0.09 (0.13)**	−0.09 (0.11)	−0.06 (0.08)	−0.01 (0.01)	−0.07 (0.08)
P	−0.06 (0.05)	−0.16 (0.11)	−0.14 (0.16)	−0.09 (0.09)	**0.00 (0.02)***	**0.03 (0.15)****
Kinsey	−0.03 (0.07)	−0.08 (0.15)	−0.15 (0.16)	−0.10 (0.10)	−0.01 (0.02)	−0.07 (0.12)
N + P[a]	−0.05	−0.07	0.98	0.71	−0.01	−0.07
Year 3 vs. Initial						
Control	0.00 (0.04)	−0.04 (0.12)	0.05 (0.09)	0.04 (0.07)	0.01 (0.02)	0.08 (0.10)
N	0.00 (0.04)	−0.02 (0.13)	**0.07 (0.15)**	**0.05 (0.11)**	−0.01 (0.02)**	−0.05 (0.12)**
Lime	**0.02 (0.04)**	**0.04 (0.12)**	0.05 (0.13)	0.04 (0.10)	0.01 (0.01)	0.08 (0.08)
$CaCl^2$	**−0.02 (0.05)**	**−0.04 (0.09)**	0.00 (0.13)	0.01 (0.09)	0.01 (0.01)	0.11 (0.09)
P	−0.03 (0.06)	−0.09 (0.14)	−0.02 (0.18)	0.00 (0.12)	**0.03 (0.01)****	**0.20 (0.09)****
Kinsey	−0.02 (0.05)	−0.04 (0.16)	−0.03 (0.17)	−0.01 (0.12)	0.01 (0.02)	0.08 (0.13)
N + P[a]	0.15	0.24	0.28	0.2	−0.04	−0.21

[a] Treatment only applied at site OSU.
* Significant change in nutrient level relative to the control treatment ($p \leqslant 0.05$).
** Significant change in nutrient level relative to the control treatment ($p \leqslant 0.01$).

mean decrease in Ca associated with $CaCl_2$ treatment was strongly influenced by one site, and $CaCl_2$ actually increased soil Ca at 7 of 10 sites by an average of 40%. The magnitude and durability of soil Ca increases were greater for the lime treatment compared to the $CaCl_2$ treatment (data not shown). Dramatic and significant increases in soil P were observed for the P treatment, which also caused relatively larger increases on sites closer to the coast with lower initial levels of soil P (data not shown).

3.2. Foliar chemistry

Initial levels of several foliar nutrients were correlated with distance-from-coast. In general, Ca, K, Mg, P, B and Mn increased with distance-from-coast, and N and N:Ca decreased (Table 6).

N and P were the only treatments to significantly change foliar nutrient levels over the 3-year study, either as absolute change in concentration or as relative change from initial concentration (Table 7). Treatment effects on foliar Ca were not statistically significant ($\alpha = 0.05$) and were difficult to interpret due to concurrent changes in foliar Ca in the control treatment at many sites and inconsistent treatment effects.

One year after fertilization, the N treatment significantly increased foliar N, but this effect was not significant after 3 years. Increases in foliar N for the N treatment were highest on the most inland sites (data not shown), which had relatively lower initial levels of foliar N. The N + P treatment increased N substantially, but the significance of this change could not be tested because it was only applied at one site.

After 3 years, the P treatment resulted in the largest and only significant increases in foliar P relative to initial levels (average proportional increase 20%). The N treatment resulted in a statically significant decrease in P concentration.

Changes in some foliar micronutrient levels were observed during the study, and the direction of change (increase or decrease) was often similar across sites and treatments, including the control (data not shown). However, the Kinsey blend was associated with a significant pulse in B one and 3 years after treatment ($p < 0.001$).

3.3. Infection levels by site

Infection index varied across sites, and differences in infection index were most pronounced for 2-year-old needles (Fig. 3). Negligible disease was detected for both needle age classes at three sites (CTC, GPH and OSU), while moderate to severe disease levels were detected at the remaining sites. The most severely impacted site (GDH) had a high infection index for both needle age classes, whereas all other sites had low levels of infection (median infection index <5%) for 1-year-old needles.

3.4. Linear mixed effects analyses: differences in infection index by treatment across sites

Treatments did not significantly affect infection index of 1- or 2-year-old needles across sites (p-values 0.47 and 0.14, respectively; Table 8). The unfertilized control exhibited the highest infection in-

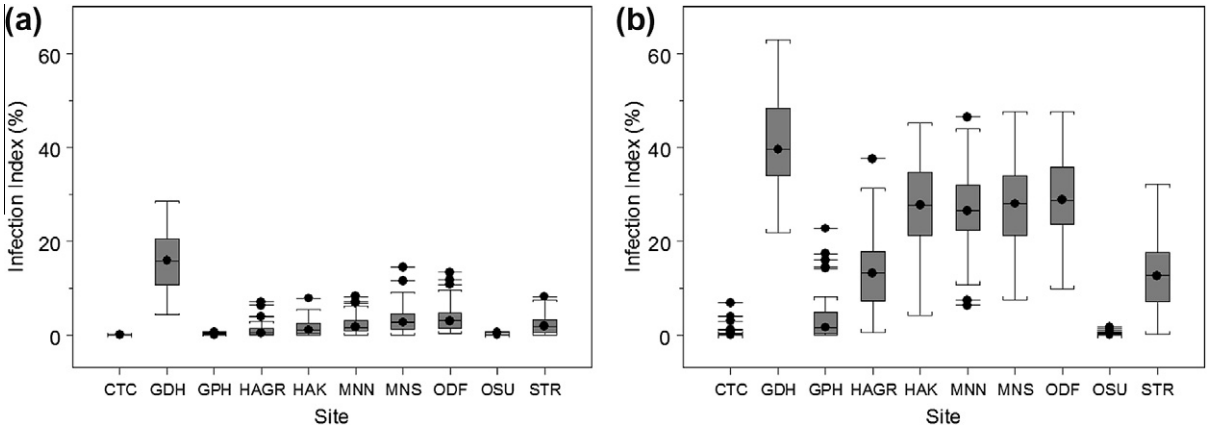

Fig. 3. Swiss needle cast infection index by site for (a) 1-year-old needles and (b) 2-year-old needles collected in May 2010, three growing seasons after fertilization treatment. Boxplot midlines represent the median, top and bottom box limits represent the 75th and 25th quartiles, and whiskers span 1.5 times the inter-quartile range.

Table 8
Swiss needle cast infection index across sites by needle age class from the Douglas-fir fertilization experiment.

Needle age	Mean[a]	Median[a]	sd[a]	*n*	Min[b]	Max[b]	*p*-Value[c]	Treatment ranks[c] (highest to lowest infection index)
1	3	0.9	5.2	565	2.7	3.3	0.47	Control, P, CaCl2, N, Kinsey, lime
2	18	17.5	15.2	555	16.7	19.9	0.14	Control, N, Kinsey, $CaCl_2$[*], lime[*], P[*]

[a] Mean, median and standard deviation (sd) summarize tree-level infection indices (*n* samples).
[b] Min and max summarize treatment-level infection indices.
[c] *P*-values for treatment effect and treatment ranks are based on linear mixed effects analyses with site as a random block effect.
[*] Significant difference in infection index between individual treatments and the control at $\alpha = 0.05$.

Table 9
Swiss needle cast infection index by site and needle age class from the Douglas-fir fertilization experiment.

Site[a]	Needle age	Mean[b]	Median[b]	sd[b]	*n*	ntrt[c]	Min[d]	Max[d]	*p*-Value[e]	Treatment ranks[f] (highest to lowest infection index)
GDH	1	15.7	15.8	6.7	58	6	11.8	19.1	0.11	1,4,5,2,6,3
	2	40.6	39.3	9.3	51		35.6	49	0.09	4,1,2,6,5,3
ODF	1	3.8	3	3	56	6	2.5	5.7	0.24	5,4,2,3,1,6
	2	29.4	28.7	8.4	56		25.7	31.3	0.78	4,1,2,3,5,6
HAK	1	1.6	1.1	1.6	55	6	0.9	2.6	0.13	6,3,4,1,2,5
	2	27.5	27.6	9.6	55		24.6	35	0.13	6,1,4,5,3,2
MNS	1	3.4	2.7	2.9	56	6	2	5.4	0.11	1,5,4,2,6,3
	2	27.3	28	9.2	54		20.1	30.7	0.49	1,2,6,4,3,5
MNN	1	2.4	1.7	2	57	6	1.6	3.5	0.37	2,1,3,6,5,4
	2	27.1	26.4	9	56		22.1	32.2	0.27	2,1,4,3,5,6
HAGR	1	1.1	0.4	1.4	59	6	0.5	1.8	0.36	6,1,4,5,2,3
	2	13.6	13.2	7.8	60		10.9	17.7	0.49	1,6,3,2,5,4
STR	1	2.1	1.8	1.9	54	6	1.2	2.9	0.48	2,3,5,6,4,1
	2	13	12.7	7.5	55		10.8	15.7	0.67	2,1,6,4,3,5
GPH	1	0.1	0	0.2	46	5	<0.1	0.1	0.37	5,3,2,4,1
	2	3.7	1.6	5.2	46		2.8	5.1	0.91	1,4,5,3,2
CTC	1	<0.1	0	0.1	57	6	0	0.1	0.19	6,2,4,5,3,1
	2	0.6	<0.1	2.1	56		<0.1	2.4	0.14	6,2,5,1,4,3
OSU	1	<0.1	0	0.1	67	7	0	0.1	0.55	5,2,3,7,1,4,6
	2	0.1	0	0.3	66		<0.1	0.2	0.21	7,5,4,1,2,3,6

[a] Sites ordered from highest to lowest mean infection index on 2-year-old foliage.
[b] Mean, median and standard deviation (sd) summarize tree-level infection indices (*n* samples).
[c] Ntrt is the number of treatments (trts) compared at each site.
[d] Min and max summarize treatment-level infection indices.
[e] ANOVA *p*-values test the null hypothesis of no treatment effect at each site.
[f] Treatment ranks are based on ANOVA beta coefficients. Trts: (1) control, (2) N, (3) lime, (4) $CaCl_2$, (5) P, (6) Kinsey, and (7) N:P.

dex for both needle age classes; slight but statistically significant differences ($\alpha = 0.05$) in infection index were detected in mixed-effects comparisons between individual treatments and the control (indicated by [∗]; right column Table 8). Across all sites, differences in estimated mean infection index between the highest- and lowest-ranked treatments (Max–Min) was only 0.6% for 1-year-

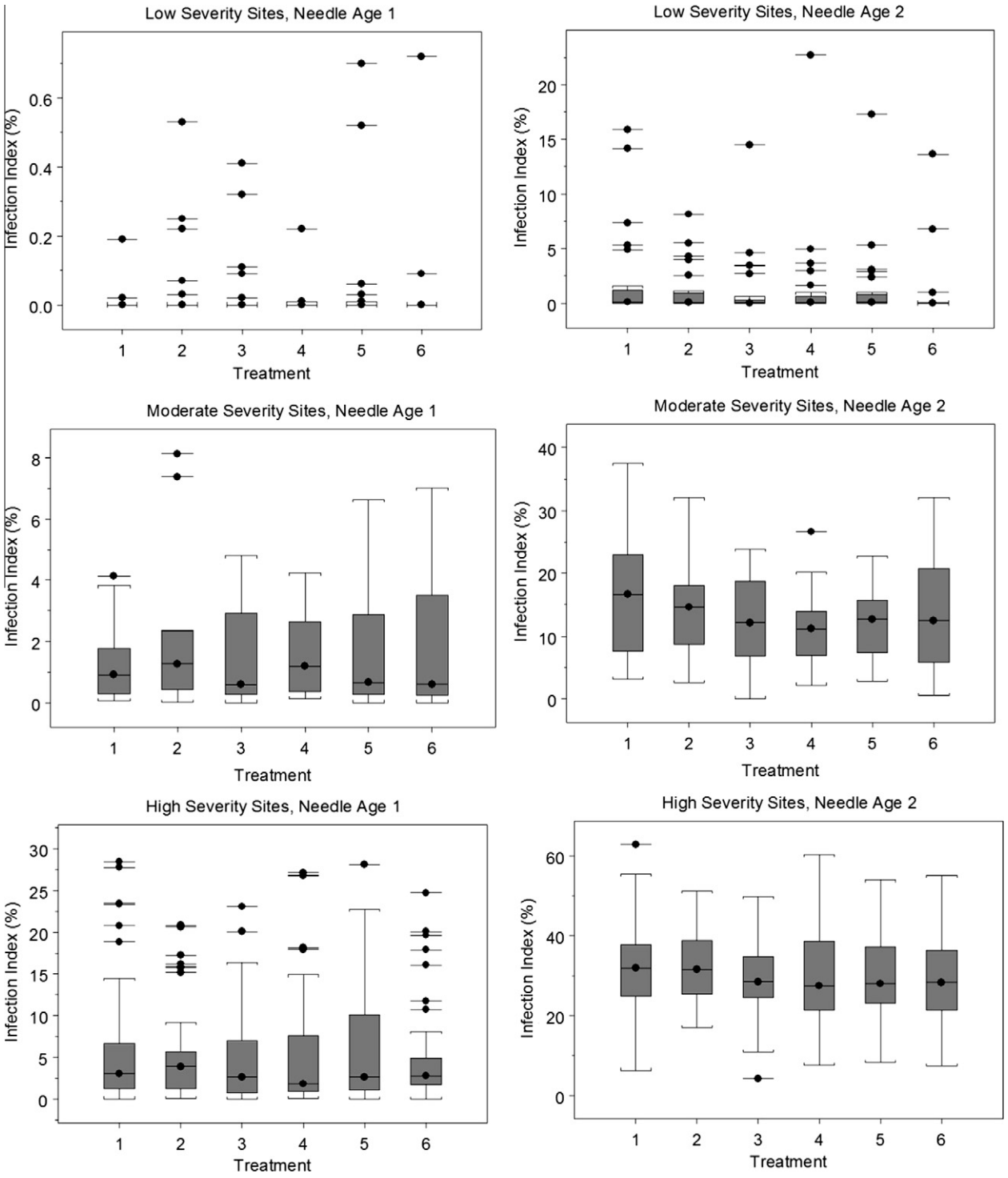

Fig. 4. Swiss needle cast infection index by treatment (trt) and each needle age class for sites grouped by disease severity level (low, moderate, high). Low = OSU, CTC, GPH; moderate = STR, HAGR, and high = GDH, HAK, MNN, MNS, ODF. Each graph depicts infection indices for 7–10 trees/trt/site for 1- or 2-year-old needles. Trts: (1) control, (2) N, (3) lime, (4) $CaCl_2$, (5) P, and (6) Kinsey. Boxplot midlines represent the median, top and bottom box limits represent the 75th and 25th quartiles, and whiskers span 1.5 times the inter-quartile range.

old needles and 3.2% for 2-year-old needles, representing negligible differences in infection between treatments (Table 8).

3.5. One-way ANOVA: differences in infection index by treatment within sites

No significant treatment effect on infection index was detected within individual study sites (all p-values > 0.05; Table 9). Differences in infection index between treatments, within sites, were generally small in magnitude, and ranking fertilization treatment from highest to lowest infection index (according to one-way ANOVA beta coefficient estimates) revealed no consistent trends in treatment-rank across sites or needle age classes (Table 9). Treatment-ranks also differed between needle age classes at many sites.

Although there was no evidence of treatment effect on disease severity, differences in mean infection index between the best- and worst-ranked treatments at individual sites were greatest for 2-year-old needles and on sites with high disease pressure that

Table 10
Linear relationships between infection index of 2-year-old needles (collected May 2010) and foliar nutrient levels pre- and post-treatment in the Douglas-fir fertilization experiment.

Nutrient		Pre-treatment (2006)	Post-treatment (2009)
N:Ca	*p*-Value	0.00	0.00
	Non-0 slope	0.16	0.12
	R^2	0.81	0.87
%Ca*	*p*-Value	0.00	0.00
	Non-0 slope	0.00	0.00
	R^2	0.86	0.89
%N	*p*-Value	0.22	0.15
	Non-0 slope	0.31	0.25
	R^2	0.18	0.24
%K*	*p*-Value	0.01	0.00
	Non-0 slope	0.00	0.00
	R^2	0.58	0.65
%Mg*	*p*-Value	0.05	0.01
	Non-0 slope	0.01	0.00
	R^2	0.39	0.58
%P*	*p*-Value	0.02	0.01
	Non-0 slope	0.00	0.00
	R^2	0.53	0.56
B ($\mu g\ g^{-1}$)*	*p*-Value	0.00	0.02
	Non-0 slope	0.00	0.00
	R^2	0.73	0.51
Mn ($\mu g\ g^{-1}$)*	*p*-Value	0.01	0.00
	Non-0 slope	0.00	0.00
	R^2	0.63	0.83

* Signifies nutrients that had a significant linear relationship with infection index (at $\alpha = 0.05$)

experienced relatively higher variance in infection index (Table 9). The most severely impacted site (GDH) displayed the greatest difference in mean infection index between the best- and worst-ranked treatments, which corresponded to lime and $CaCl_2$, respectively. When sites were grouped by relative disease severity level (low, moderate and high), graphical representations of infection index by treatment also supported a lack of treatment effect (Fig. 4).

3.6. Simple linear regression: correlation between site-level infection and nutrient levels

Linear regression models of infection index of 2-year-old needles and foliar nutrient levels measured before and 3 years after treatment provided evidence that Ca, K, P, B, and Mn were negatively correlated with infection index (Table 10). Scatter plots revealed correlation between these factors and distance-from-coast (not shown). There was no evidence of a linear relationship between disease severity and foliar N or the ratio of N:Ca, as indicated by linear regression *p*-values > 0.05 and lack of evidence for a non-zero regression slope, respectively. There was no evidence that fertilization treatments influenced disease severity levels in this study. These correlative trends between foliar nutrient levels and infection index are believed to be linked to climatic and topographic variables that covary with soil and foliar nutrient levels in the study region and are known to strongly influence the causal fungus.

4. Discussion

The potential effects of fertilization on SNC severity in western Oregon is of great interest to owners of Douglas-fir timberland, with respect both to possible deleterious effects of conventional nitrogen fertilization and to possible ameliorating effects of other nutrients. This study found no evidence that fertilization treatments applied at the levels tested significantly altered SNC severity, as measured by the abundance of fruiting bodies on needles. Disease severity did not differ between treatments within or among the ten stands examined, even though they covered a wide range of initial disease severity levels, soil nutrient content, and other site attributes. The hypotheses that N fertilization increases susceptibility to SNC and that Ca and alternative fertilization regimes decrease susceptibility were not supported by the 3-year results from this field trial.

Ground-applied mineral nutrients must infiltrate the soil, remain in available form, be absorbed by the roots, and be transported to the foliage before potential effects on a foliar pathosystem are likely to be realized or detected. Foliar chemistry assessments showed that most foliar nutrient levels were not significantly altered by fertilization treatment. Fertilization effects on foliar chemistry differed by site, and were significant only for the P (increased P), N (increased N and decreased P) and Kinsey (increased B) treatments. Fertilization effects on soil chemistry were evident for the P (increased pH and P), Kinsey (increased pH and Ca) and lime (increased pH and Ca) treatments. It was important to report that some treatments measurably and significantly changed foliar and/or soil chemistry, particularly in the absence of treatment effects on disease severity.

There was no significant difference in infection index between treatments within sites. Likewise, there was no evidence that infection index differed between treatments when all data were pooled across sites. Although some differences in infection index were detected when individual treatments were directly compared to the control treatment, which had the highest infection index when all sites were pooled, the magnitude of these differences were negligible and are not believed to be biologically or economically significant. In contrast, there were large differences in mean infection index between sites. At the site with the highest SNC severity, approximately 40% of the stomata on 2-year-old needles were occluded by pseudothecia, compared to 0.1% at the lowest severity site.

Topographic and climatic factors that covary with nutrient levels and disease severity are known to strongly influence the causal fungus. Site-level comparisons supported previously observed correlations between disease severity, as measured by foliage retention, and levels of several foliar nutrients (e.g., Ca) (Perakis et al., 2005), but did not support a relationship between infection levels and foliar N or the ratio of N:Ca. Perakis et al. (2005) observed foliar N levels from 0.85% to 1.74% in Douglas-fir stands of the Oregon Coast Range. A similar range was observed in this study before fertilization treatment (1.21–1.81%), and the highest level of foliar N achieved through fertilization was 2.36% (N + P treatment).

In contrast to our findings, the study conducted by El-Hajj et al. (2004) reported significantly increased (2.2- to 3.6-times higher) pseudothecia density on 2-year-old needles of urea-treated trees compared to control trees. Urea fertilization had resulted in foliar N levels of 1.0–1.30%, compared to 0.9–1.0% for the control treatment. This study was conducted on 10-year-old Douglas-fir trees that were part of a progeny test of low-elevation, open-pollinated seed sources at an experimental forest in Priest River, ID. The progeny test site was located at approximately 700 m elevation and needle retention averaged 2.7 years (El-Hajj et al., 2004). It is unclear why this study obtained markedly different results than our experiment, particularly because increases in foliar N were not dramatic and apparently did not exceed the 1.4% threshold for N-limitation in coastal Douglas-fir (Perakis et al., 2005). There are several reasons that we urge caution in extending these results to coastal Douglas-fir stands of western Oregon, including the

small sample size (5 trees/treatment); the lack of replication across stands; the younger tree age; the interior host subspecies (*P. menziesii* ssp. *glauca*); the repeated and intensive fertilization required to raise foliar N; and the inland and high-elevation location of the study.

While the levels of fertilizer applied did not affect disease severity in our study, some did directly stimulate tree growth on these same sites (unpublished: Mainwaring et al., 2009; Mainwaring and Maguire, 2010). After accounting for site and tree covariates, N and lime fertilization resulted in marginally greater volume production (~3.4%) compared to the control treatments across sites. The relative benefits of fertilization varied by site. For example, significant growth responses to N treatment occurred on sites with relatively lower site indices and higher soil pH. Site indices less than about 33.5–36.5 m (110–120 ft; high site III to low site II) at age 50 are considered moderate for Douglas-fir in the Coast Range (King, 1966). Ideally, managers should assess soil and/or foliar chemistry in their stands in order to select the most appropriate fertilization regimes, but the diagnostics for identifying stands that are likely to respond are poorly understood. More work on this topic would help timber growers to avoid the expense of fertilizer applications that are unlikely to result in increased volume growth given the initial site conditions. While fertilization treatments have the potential to positively or negatively affect Douglas-fir volume growth in stands impacted by Swiss needle cast, impacts on growth are apparently unrelated to fertilization effects on the host–pathogen interaction.

Studies that have been conducted in the Pacific Northwest and New Zealand on the influence of silvicultural strategies (commercial and pre-commercial thinning and vegetation management) on volume growth and SNC severity have also found no direct interaction between silvicultural regime and disease severity (Hood and Sandberg, 1979; Mainwaring et al., 2005; Shaw et al., 2011). In other words, various silvicultural regimes did not alter the host–pathogen interaction, but instead conferred the same relative benefits as in the absence of disease. This may be due to the specific mechanisms of infection and colonization in this pathosystem, which do not appear to be significantly affected by changes in host resource availability, vigor or nutritional status that might be expected under operational silvicultural treatments. There is no evidence that inducible or constitutive host defense responses play a role in this pathosystem. If present, these defense responses might provide a basis for altered host nutritional status to directly affect host susceptibility, as has been observed in other agricultural and forest pathosystems (e.g., *D. pinea*) (Stanosz et al., 2004; Blogett et al., 2005; Sugimoto et al., 2010; Wallis et al., 2011). Thinning and other silvicultural activities also have the potential to influence the infection environment; however, on sites close to the coast with high levels of disease, it is believed that prolonged leaf wetness during the infection period in spring and summer exceeds a threshold beyond which thinning practices have little impact.

An improvement on our study design would be to measure nutrient levels individually for all trees (not pooled by treatment), which would provide a more robust sample size and would make it possible to conduct regression analysis for all sample trees with infection index as the response variable and nutrient levels or ratios as explanatory variables. However, the goal of this study was to determine if and how specific operational and experimental fertilizer applications, not foliar or soil nutrient levels per se, impact disease severity and tree growth, and the study design accomplished this objective. A longer-term experiment with repeated fertilizer applications and several disease severity assessments could also help to evaluate pathogen–nutrient dynamics over time. Fertilization treatments at the rates applied in this experiment did not affect the density of fungal fruiting bodies on the foliage of Douglas-fir trees.

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