AN ABSTRACT OF THE THESIS OF

Gabriel A. Crane for the degree of Master of Science in Forest Science presented on February 5, 2002. Title: Effects of Fertilization, Vegetation Control, and Sulfur on Swiss Needle Cast and Growth of Coastal Douglas-Fir Saplings

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A series of studies and replicated field sites were implemented in the Oregon Coast Range within Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) sapling plantations infected with varying levels of Swiss needle cast (SNC) caused by the fungus, *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*). This research was conducted to understand the effects of fertilization, vegetation control, and elemental sulfur on *PG* infection and subsequent growth in Douglas-fir saplings. Also, quantum yield (measured via chlorophyll fluorescence) was evaluated as a means to determine *PG* infection level differences on an individual tree basis.

The removal of competing vegetation on all study sites had a positive effect on mean diameter at breast height (DBH) growth (16% to 19%). No significant differences in height growth based on vegetation treatments were documented. Fertilizer treatments had no significant impact on height or DBH growth. Vegetation control and fertilization as individual treatments had no significant

impact on *PG* infection. Vegetation removal increased foliar nitrogen concentration at each site by 4.3%, 4.5%, and 8.4%, respectively. Over all sites there were no consistent foliar nutrient concentration responses to fertilization except for boron.

Elemental sulfur (Thiolux®) applied as a foliar application with and without TacTic® sticker at a rate of 25 lbs/100 gal of water (8 oz sticker/100 gal) as well as a Thiolux® ground and a foliar Bravo® (chlorothalonil) application were applied to individual Douglas-fir saplings. Bravo® applied at a rate of 3.75 pts/100 gal of water resulted in a significant reduction in *PG* infection when compared to all other treatments. The Thiolux® with-sticker was also significantly effective at lowering *PG* levels when compared to the control treatment, but 10 times less effective than the Bravo® treatment. Foliar treatment applications of Thiolux® with and without sticker led to significantly increased levels of foliar sulfur by 86% and 57%, respectively. Height and DBH growth were not significantly affected.

Quantum yield was measured on one group of Douglas-fir saplings (sulfur study) and on one group of Douglas-fir seedlings (Douglas-fir potted seedling study) with varying levels of *PG* infection using a modulated chlorophyll fluorometer. No significant differences in quantum yield were found among respective treatments in the sulfur study. There was a significant relationship between pseudothecia density (%) and quantum yield in the Douglas-fir potted seedling study. As the percent pseudothecia increased, there was a decrease in quantum yield values (R^2=.43).

Effects of Fertilization, Vegetation Control, and Sulfur on Swiss Needle Cast and Growth of Coastal Douglas-Fir Saplings

by

Gabriel A. Crane

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Gabriel A. Crane, Author

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This research and paper benefited greatly from the contribution of several individuals.

Chapter 2: Dr. Robin Rose, Scott Ketchum, and Diane Haase were instrumental in the experimental design, interpretation of results, and editing. Dr. Jeff Stone and Wendy Sutton were responsible for the quantitative PCR.

Chapter 3: Dr. Robin Rose, Scott Ketchum, and Diane Haase were instrumental in the experimental design, interpretation of results, and editing. Dr. Jeff Stone and Wendy Sutton were responsible for the quantitative PCR.

Chapter 4: Dr. Dan Manter was instrumental with measurements and analyses, as well as pseudothecia counts. Diane Haase provided valuable technical assistance. Dr. Robin Rose, Scott Ketchum, and Diane Haase all collaborated on edits of the manuscript.

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Effects of Fertilization, Vegetation Control, and Sulfur on Swiss Needle Cast and Growth of Coastal Douglas-Fir Saplings

Chapter 1

General Introduction and Literature Review

by

Gabriel A. Crane

1.0 Introduction

Rapid successful establishment of forest tree seedlings has been a major goal for decades on private, state and federal lands in the Pacific Northwest. Vast improvements in seedling quality, through better nursery practices and early vegetation control after outplanting have led to improved reforestation success in the region. However, the increasing incidence of Swiss needle cast (SNC), caused by the fungus *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*), in coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) threatens established pure plantations of this species as well as future reforestation efforts with Douglas-fir.

SNC and the fungus that causes the disease, were first described in 1925 as devastating Douglas-fir plantations in Switzerland (Gaeumann, 1930). From Switzerland the disease spread to Germany, Austria, and Czechoslovakia, generally following the prevailing winds. It was first reported in the United States in 1939, although herbarium records provide evidence of the disease as early as 1916 in California (Morton and Patton, 1970).

In response to the SNC epidemic, a survey was conducted in Oregon and Washington in 1938 (Meinecke, 1939). The fungus was found on older, senescing needles throughout the Douglas-fir range. However, the disease was not associated with the fungus throughout the entire survey area. Disease is defined in this paper as a "sustained disturbance to the normal function or structure of a tree as provoked by biological (biotic), chemical, or physical (abiotic) factors of the environment"

(Filip *et al.*, 1994). It is important to keep in mind that disease is a product of three interacting factors: -- the pathogen, the host or tree, and the environment (Hansen, 1996).

SNC is now known to be present in a number of states where Douglas-fir is present, including Oregon, Washington, California, Rhode Island, New Hampshire, Vermont, Michigan, and Wisconsin (Michaels and Chastagner, 1984). SNC also is widespread in Europe, Japan, and New Zealand (Morton and Patton, 1970). *PG* is believed to be endemic to the Pacific Northwest.

In the mid – 1970s Christmas tree growers in the Pacific Northwest began reporting serious crop damage due to SNC that resulted in annual losses of $3.4 million (Michaels and Chastagner, 1984). In the late 1970s and early 1980s, severe SNC was reported in a few forest plantations in Oregon and Washington. However, much of this damage was attributed to bad seed sources or to localized environmental conditions that favored disease development. *PG* was considered a good example of a common, but benign forest pathogen that only becomes damaging on off-site trees and in altered environments (Hansen *et al.*, 2000). Since the late 1980s SNC has become increasingly severe on an annual basis in forest plantations and naturally established stands in the coastal regions of Oregon and Washington. The most severe damage is seen along the north coast of Oregon within 15 miles of the coastline in an area referred to as the fog belt, roughly corresponding to the Sitka spruce zone (Franklin and Dyrness, 1973).

Swiss needle cast is now associated with a chronic decline in the health of Douglas-fir. Symptoms are most obvious in plantations younger than 30 years of age. Severely infected stands appear noticeably yellow to tan-yellow when viewed from a distance. Discoloration is most visible from late winter until shortly after bud break in the spring. Southerly aspects usually display more severe symptoms than other aspects, due in part to drier soil conditions and more exposure to afternoon heat (Manter, 2001). Damage caused by SNC includes needle chlorosis, premature needle loss, and an associated reduction in photosynthesis (Hood, 1982). Primary shoot needles are lost more readily than are secondary shoot needles (Hood *et al.*, 1990). Diseased trees often lose all but the current year's complement of needles in contrast to the four-year complement retained by healthy Douglas-fir. The actual mechanism by which the disease induces these symptoms is not completely understood (Hood *et al.*, 1990), but relates to physiological disturbance of photosynthetic mechanisms (Manter, 2001).

The rate of spread of SNC is slow in comparison to other wind-borne fungal diseases (Hood *et al.*, 1990). This is believed to be due to the low rate of reproduction (one infection cycle per year) of *PG*, limited dispersal, and slow establishment of infection (Hood *et al.*, 1990). No asexual repeating phase has yet been confirmed.

Little is known about the factors that promote or hinder the spread of SNC. Research suggests that environmental factors, which alter water regulation or needle physiology, may affect needle loss on diseased trees. Supporting evidence

includes observations that needle loss usually is greater on parts of the tree exposed to wind and sun, rather than on the lower crown.

Very little is known about the relationships between *PG* infection levels and Douglas-fir sapling growth when fertilization, vegetation control, and sulfur treatments are implemented. This information may prove to be valuable to forestland owners so that successful regeneration and growth of sustainable Douglas-fir plantations is maintained.

To my knowledge, there has been no published literature on chlorophyll fluorescence and the quantification of *PG* infection levels on an individual tree basis. This could prove useful to forestland owners by allowing an efficient, consistent, and objective quantification of *PG* infection.

The overall objectives of this research were to identify the effects of fertilization, vegetation control, and sulfur treatments on SNC disease severity and the subsequent growth of coastal Douglas-fir saplings. Additionally, quantum yield (measured via chlorophyll fluorescence) was used for measuring the photosynthetic activity of individual trees as an index of differences in SNC disease severity.

Following this introduction is a general literature review describing *PG*, the ascomycete causing SNC of Douglas-fir. The current state of knowledge about the infection biology of *PG* is also reviewed.

Chapter two describes the effects of fertilization and vegetation control on SNC and growth of coastal Douglas-fir saplings. Three fertilizer treatments and two vegetation control treatments were used to explore the effects on *PG* infection

and growth. Chapter three describes the effects of Bravo® (chlorothalonil) and elemental sulfur (Thiolux®) on SNC and growth of coastal Douglas-fir saplings. Five separate tank mixes were applied in order to test for *PG* infection and growth differences. Chapter four evaluates the usefulness of quantum yield in detecting differences in *PG* infection levels on an individual tree basis. The fifth and final chapter summarizes the overall thesis conclusions.

1.1 Literature review

The purpose of this literature review is to become familiar with the biology of *PG*. Additionally, influences on SNC will be reviewed.

PG, the ascomycete causing SNC of Douglas-fir, parasitizes its host by utilizing the products of photosynthesis (Hansen, 1996). In diseased trees, it reduces assimilation rates of the foliage and impairs needle function to the point that chlorosis develops (Hansen, 1996).

PG damages Douglas-fir needles by interfering with stomatal function. *PG* reduces gas exchange (H_2O and CO_2) by apparent blockage of needle stomata (Manter *et al.*, 2000). Stone and Carroll (1986) found that *PG* produces dense mats of hyphae in stomatal antechambers and pseudothecia at stomatal openings. It does not penetrate cells but grows within the mesophyll and on the leaf surface by absorbing water and nutrients from the intercellular spaces (Capitano, 1999). Additionally, Manter *et al.* (2000) report that maximal stomatal conductance and

net assimilation rates are inversely proportional to the number of stomata occluded with pseudothecia. In other studies, Michaels and Chastagner (1984) observed significant water loss in infected trees when compared to healthy trees.

PG can be seen on the underside of needles as rows of black fruiting bodies (pseudothecia). In late winter and spring pseudothecia emerge from the stomata of the previous year's needles. *PG*, with bi-tunicate asci and two-celled ascospores, can be distinguished from a similar fungus, *Rhizosphaera*, which has one-celled conidion spores (and no asci). Production of ascospores can continue for up to six months (Hansen, 1996). Based on research data from Michaels and Chastagner (1984), one-year-old needles have the potential to release 10 times as many ascospores as do two-year-old needles. Ascospores tend to be released at temperatures of 5-30° C and in greatest abundance at 20° C (Michaels and Chastagner, 1984).

PG requires water on the needle surface for spore release, germination, and penetration. Infection is generally higher where fog during the period of shoot elongation is greatest. Temperature and needle surface moisture may also affect the rate of vegetative growth of the fungus within and on needles.

The environment of the Oregon Coast Range is ideal for *PG*. A long growing season with high rainfall, fertile soils, and moderate temperatures result in a lengthened period of susceptibility to *PG*. Coastal summer fogs also contribute to greater moisture on needle surfaces, which is necessary for infection to occur.

The characterization of provenance variation in resistance to *PG* is important to the understanding of this pathosystem and may point to the use of natural resistance in the control of this disease (McDermott and Robinson, 1989). McDermott and Robinson (1989) found that provenances from locations with higher rainfall, and presumably higher selective pressure for disease resistance, expressed higher resistance to SNC. Hood (1982) observed that mean *PG* infection varied regionally in southern British Columbia, and this variation fitted recognizable climatic patterns. A significant regional correlation was found between mean infection and approximate mean rainfall during the May – July period (Hood, 1982). Based on observations from Hood (1982) and McDermott and Robinson (1989), an implication can be drawn that there appears to be resistance to endemic levels of SNC in natural populations of Douglas-fir. This implication is based on regional levels of disease pressure.

Therefore, genetic resistance may be an important ingredient in controlling the spread and severity of SNC in the Oregon Coast Range. The correlation of geographic variation in traits with selective factors is the most common and oldest method of inferring natural selection (Endler, 1986).

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Chapter 2

Effects of Fertilization and Vegetation Control on Swiss Needle Cast and Growth of Coastal Douglas-fir Saplings

by

Gabriel A. Crane

2.0 Abstract

This study was initiated in May 1999 to determine if applied silvicultural treatments (fertilization and vegetation control) could enhance the growth of Swiss needle cast (SNC) affected Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) saplings and/or alleviate *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*) infection. Current results indicate that the removal of competing vegetation on all study sites has a significant positive effect on mean DBH growth (16% - 19%) when compared to the non-removal treatment. However, no significant differences in height growth based on vegetation treatments were documented. Fertilizer treatments had no significant impact on height or DBH growth. There was a significant interaction between fertilizer and vegetation control at the Bushy Peterson site with regard to *PG* infection. No significant differences with regard to *PG* infection levels were found at the other two respective sites. Vegetation removal at the Charlie Olson site exhibited significant differences in foliar nitrogen (8.4% increase) when compared to the non-removal treatment. The other two sites did not exhibit significant differences in foliar nitrogen, but they did demonstrate a similar trend to the Charlie Olson site in that their foliar nitrogen levels were increased by 4.3% and 4.5%, respectively. At all sites there were no consistent foliar nutrient concentration responses to fertilization other than for boron.

2.1 Introduction

There are currently over 250,000 acres of Swiss needle cast (SNC) affected Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands within the Oregon Coast Range. Vegetation control and fertilization and/or their interactions in altering *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*) infection and increasing biomass growth are of considerable theoretical and practical interest. Vegetation control is a basic concern in forest stand management (Opio *et al.*, 2000). Both intraspecific and interspecific competition can decrease resource availability, thereby reducing tree growth in comparison with that of trees without competition (Cole and Newton, 1987). Forest scientists have stressed the need to understand the interactions between forest trees and soils as a prerequisite for high and sustained production of timber over wide ranges of the globe (Mahendrappa *et al.*, 1986). It has been demonstrated that changes in nutrient element dynamics due to fertilization can have a significant impact on biomass production and overall growth (Mitchell *et al.*, 1996).

In a warm, low summer rainfall climate, the primary effect of weed control is to conserve soil moisture and reduce tree moisture stress (Preest, 1977). Preest (1977) showed that there was a strong positive correlation between available soil moisture and weed control and a strong negative correlation between tree moisture stress and available soil moisture. High moisture stress is known to adversely affect photosynthesis and tree growth. Conceptually, weed control around SNC-

affected trees should increase tree growth because of increased soil moisture, decreased plant moisture stress, and increased photosynthesis compared to untreated trees affected by SNC.

Complete vegetation removal can increase nutrient cycling through more rapid decomposition of the organic horizons due to higher temperatures and moisture. The reduction in the amount of competition for resources provides a flush of nutrients. This "flush" only lasts as long as it takes the organic materials to breakdown before nutrient levels decrease to a steady state. The microbial populations associated with decomposing roots of the removed competing vegetation immobilize nitrogen (N) in the soil zone immediately adjacent to the roots (Cochran, 1968). In theory, the roots on the live trees have less competition for unoccupied soil, and thus an increased amount of N made available by vegetation removal.

Vegetation removal may affect plantation productivity by altering the efficiency with which nutrients are utilized (ANPP/unit nutrient uptake). Mitchell *et al.* (1996) observed that thinning decreased nutrient use efficiency (NUE) for all elements, however foliar efficiency (annual ANPP/unit of foliage mass) was increased through year nine following thinning. This should be expected as better water and light conditions exist after a thinning as well as a reduction in competing vegetation. Eventually, this will again decrease as the canopy closes and competition increases.

Nutrient availability is determined by both the condition of the soils and the condition of the trees. A high rate of nutrient supply can lead to a high rate of biomass production that entails "locking up" a substantial amount of nutrients in tree biomass and thus may reduce nutrient availability in the soil (Nambiar and Brown, 1997). It has been observed that a high rate of nutrient uptake (and recycling) may lead to a high rate of production if a species is moderately or highly efficient at using nutrients; alternatively, a high rate of uptake coupled with low efficiency of use could lead to poor or moderate rates of production (Nambiar and Brown, 1997).

Operational programs to improve nutrient regimes using fertilizers have been developed in forest areas throughout the world. Needs for all nutrients within a plantation are dynamic. That is, they increase with stand development, peak near crown closure, then fall back to lower levels because of internal recycling (Switzer and Nelson, 1972). Accurately interpreting the effects of nutrient cycling with regard to fertilization and long-term site productivity have historically been less of an issue based on the amount of literature published. Research dealing with fertilization in the PNW has focused on N due to its positive growth effects when applied to Douglas-fir over a wide range. There tends to be an adequate amount of soil N, but much is not accessible for plant use. If N deficiency is relieved using fertilization, nutrient uptake of other macronutrients can be increased (Mitchell *et al.*, 1996). However, with too much N fertilization, Vogt *et al.* (1985) report that

fine root production can actually decrease. This would lower the overall nutrient uptake and efficiency of a tree.

The influence of specific macro- or micro- nutrients is of varying importance for growth, as well as resistance of plants to pathogens and adverse environmental conditions (Dimitri, 1977). For example, one nutrient may increase the resistance of a plant to a particular disease, but it may reduce the resistance of another plant.

Lophodermium pinastri is an important disease of pine species that causes needle cast. Like *PG*, there are serious economic implications due to the damage caused by this fungus. Rack (1965) found no increase in resistance against infection and spreading in a trial of nutrient shortage subsequent to fertilization, but damage by needle cast was less in the fertilized plots due to increased growth. This suggests that fertilization of *PG*-infected trees may help to promote growth, even if the infection level is not lowered.

Balanced nutrition in trees to obtain growth responses and reduced disease susceptibility in forests has been recognized in past research (Durzan, 1974). It is known that fertilizer additions of specific nutrients can create imbalances of other nutrients, thus leading to reductions in growth or effects on tree health (Lambert, 1986).

The physiological role of sulfur is associated with that of N and, as a component of the amino acids cysteine, cystine, and methionine, sulfur is vital to protein synthesis (Lambert, 1986). Sulfur in excess of that required to balance N in

protein formation is accumulated as sulphate sulfur (Lambert, 1986). Foliage sulphate sulfur is therefore often used as the index of tree sulfur status.

It has been hypothesized that sulfur deficiency, possibly induced by high N levels, leads to high foliar arginine accumulations and that these can be utilized as a food source by fungal pathogens, thus causing rapid infection (Lambert and Turner, 1977). Lambert (1986) demonstrated that the level of *Dothistroma* infection was higher in N–treated plots than in control plots of *Pinus radiata*. Van den Driessche and Webber (1977) found that nitrogenous fertilizers increased arginine concentrations in Douglas-fir. Thus, with a more abundant food source (arginine) fungal pathogens such as *PG* can more rapidly colonize their host and increase overall infection levels. Turner *et al.* (1977) showed that N fertilization rapidly utilized foliar sulphate sulfur. In this situation, the combination of decreased sulfur and increased N levels create an environment that is favorable for increased arginine levels and thus more severe fungal colonization.

Based on the aforementioned research, the hypothesis can be made that where foliage sulfur levels are adequate and in the absence of other environmental stress, foliage fungal infection will usually be low. In the case of the foliage sulphate sulfur level being adequate, moderate to low application rates of N fertilizer result in improved volume growth without increased susceptibility to foliage infection in *P. radiata* stands (Lambert, 1986). The consideration that high levels of N usually increase the susceptibility of plants to both obligate and facultative parasites has previously been proposed (Hesterberg and Jurgenson,

1972). Similarly, high N levels may be linked to increased tissue succulence or to a reduction in the level of some metabolite that inhibits the pathogen.

The objectives of this study were to determine if fertilization and vegetation control enhance the growth and vigor of Douglas-fir saplings and/or alleviate *PG* infection. The study was initiated in May 1999.

2.2 Materials and methods

Field trials were conducted at three different sites installed across an east/west transect of the Oregon Coast Range. Installation of study replicates across this gradient was chosen because the greatest level of growth loss associated with SNC has been found in the coastal regions and lowest losses eastward toward the Willamette Valley. Eastward from the coast, drier conditions are not conducive to SNC.

The site nearest the coast was located between Toledo and Siletz (South Drake). This particular site was on The Timber Company's (now Plum Creek Timber Co.) ownership. The second site was midway between the coast and the Willamette Valley near Eddyville (Bushy Peterson). This site was located on Starker Forest lands. The third site is on the western valley fringe close to Summit (Charlie Olson). This site also was on Starker Forest lands. Each site had existing six-year-old Douglas-fir plantations when the experiment was implemented.

2.3 Experimental design and treatments

At each site the experiment was a randomized complete block design with five replications (blocks) of each treatment plot. An exception to this was the Bushy Peterson site where there were only four replications due to limited space. The six treatments consist of a 2 X 3 factorial design with two levels of weed control and three levels of fertilization. Each treatment plot is 70 X 70 ft and encompasses 25-35 operationally planted trees of which the center most 15 trees were evaluated. Trees not evaluated are considered buffer trees between treatment plots. Trees with forked stems or originating from natural regeneration were not measured. Each tree was clearly identified with an aluminum tag, marking paint, and flagging. Individual plots were marked in each corner with white PVC stakes approximately one meter in height. Flagging was strung between stakes completing the plot layout.

There were two vegetation control treatments:

1) No control
2) Control of all competition for three consecutive years

All treatment plots by block were assigned at random. On September 7-9, 1999, all woody vegetation on the South Drake and Bushy Peterson sites was manually cut to ground level. The Charlie Olson site did not have a significant amount of woody vegetation and therefore did not require manual control of woody vegetation. In addition, vegetation-free plots received an herbicide application broadcast with a

backpack sprayer. Fall application of sulfometuron at 3 oz/ac and glyphosphate at 1.5 qt/ac was used to eliminate all woody and herbaceous competitors. Additional spring and fall applications of herbicide were used to maintain vegetation-free conditions.

The three fertilizer treatments were:

1) Unfertilized control
2) 400 g of 9-17-17/ tree / application
3) 400 g of 18-17-17/ tree / application

The six-month controlled-time release fertilizer formulations with minor elements included were from Simplot Soilbuilders Company and were identical with the exception of N content (Table 2.1). Applications were made initially on September 8-10, 1999 to each of the 15 trees in the plots designated for fertilization. Fertilizer blends were surface applied around the base of the tree. Fertilizer was tossed by hand into the middle of the tree at approximately 2 ft off of the ground and allowed to scatter as it fell through the branches. Fertilizer applications were made again in April and October 2000, as well as April 2001.

Table 2.1. Simplot controlled-release fertilizer formulations.

						18-17-17					
%	N	P	K	S	Ca	Mg	Zn	Fe	Cu	Mn	B
	18.4	17.2	16.8	3.4	4.2	.06	.06	.06	.02	.06	.01
						9-17-17					
%	N	P	K	S	Ca	Mg	Zn	Fe	Cu	Mn	B
	9.5	17.2	16.8	3.4	4.2	.06	.06	.06	.02	.06	.01

2.4 Measurements and sampling

2.4.1 Morphology

Initial diameter at breast height (DBH) and height were measured in May 1999. Measurements were also recorded in October 1999 and 2000. Height measurements were taken using calibrated Crain® 11 m telescoping fiberglass rods. DBH measurements were taken with Spencer® diameter tapes. Seasonal growth is calculated by subtracting the previous years growth from the current years growth. Height to diameter ratio (HDR) is an individual tree based index and is calculated by dividing the height of the tree by the DBH of the tree.

2.4.2 PG infection

An initial SNC assessment was made in mid-July 1999 on a branch at breast height. This same branch was used for continuing assessments of *PG* infection. Assessments were made on a yearly basis during May of 2000 and 2001 from the same branch where the previous assessment was taken. The assessment consisted of ocularly estimating the percentage of needle retention for each cohort of needles on the main stem of the branch and also on a lateral marked for future assessments (Figure 2.1).

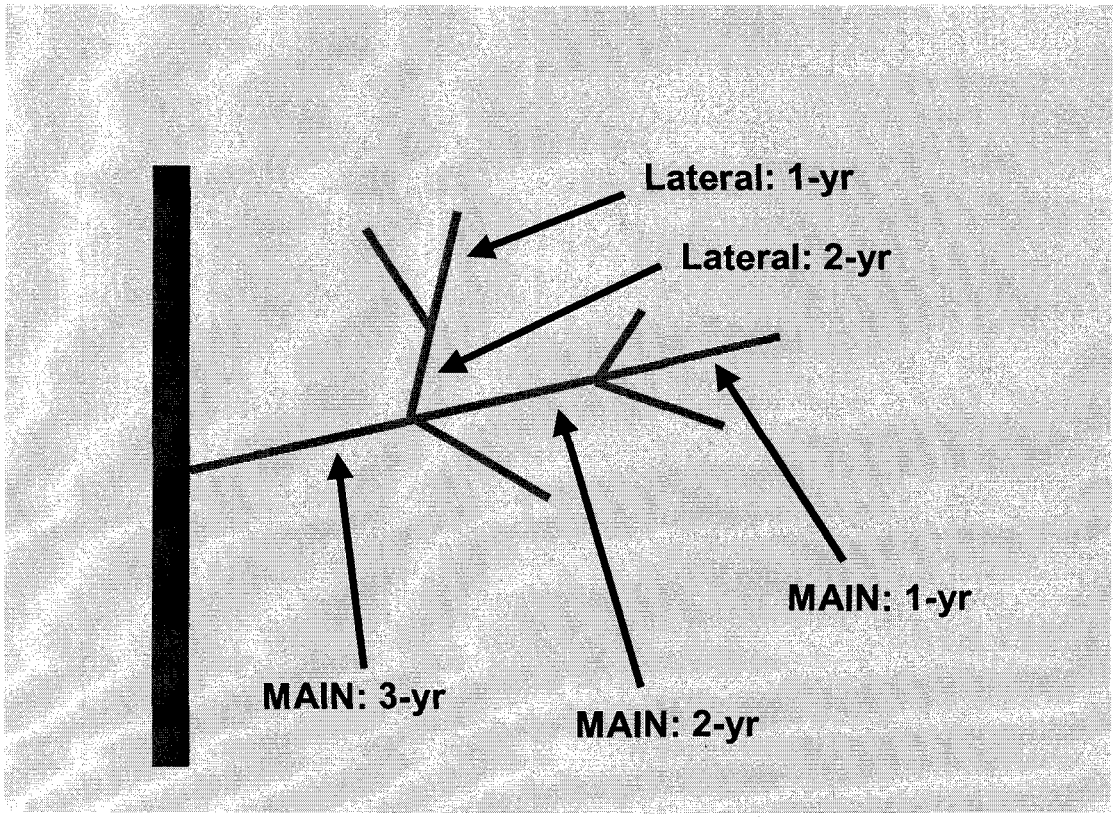


Figure 2.1. Needle cohort retention assessment diagram.

When monitoring the disease on an individual tree level, needle retention has correlated well with disease severity (Filip *et al.*, 2000). By tagging and using the same branches for monitoring purposes every year, it was possible to follow the progression or decline of the disease and its' severity.

In February 2001, current-year foliage was sampled at all sites on an individual tree basis. Five sub-samples were taken from the top one-third of the crown from each individual tree. The sub-samples were pooled and analyzed on an individual tree basis. Using the Polymerase Chain Reaction (PCR) procedure, the relative amount of *PG* fungus within needles was identified. PCR results in a ratio of picograms of *PG* DNA to nanograms of Douglas-fir DNA. PCR is being used because it has the advantage of speed, technical simplicity, low detection limits, and specificity over other procedures (Stone *et al.*, 1999). This procedure has the ability to determine the exact level of fungus within an individual needle. However, it is important to take samples from the same age class and approximate location on all sample trees in order to reduce within tree sample variation.

2.4.3 Nutrient analysis

Initial foliar (Table 2.2) and soil nutrient (Table 2.3 and 2.4) analyses were performed on samples from each site in late October 1998. One and two-year-old foliage was collected from mid-canopy on 10 trees at each site. Three samples from each individual tree were collected. All samples were pooled and a composite sample from each site was sent for analyses.

Table 2.2. Initial foliar nutrient levels (October 1998).

Site	Needle Weight (g)/200	N %	P %	K %	Ca %	Mg %	B ppm	Fe ppm	Mn ppm	Cu ppm	Zn ppm	S %
Charlie Olson	1.40	1.85	0.21	0.92	0.32	0.09	18	94	518	4	20	0.12
Bushy Peterson	1.25	1.82	0.20	1.02	0.33	0.08	19	61	371	4	18	0.13
South Drake	1.22	1.81	0.16	0.79	0.21	0.10	20	50	25	3	15	0.16

Table 2.3. Initial soil total nutrient levels (October 1998).

	P ppm	K ppm	Ca ppm	Mg ppm	Mn ppm	Fe ppm	Cu ppm	Zn ppm	Na ppm
Light Infection--Charlie Olson									
A-Horizon	1477	1754.5	2032.1	3311.8	1569.4	35754	23.8	87.0	89.6
B-Horizon	851.4	1906.2	1035.6	4054.4	1168.2	41285	27.8	97.1	87.2
Moderate Infection--Bushy Peterson									
A-Horizon	542.6	1410.6	739.1	4609.4	667.9	31305	19.2	84.5	99.0
B-Horizon	485.7	1245.3	475.6	4371.6	553.0	37954	23.1	85.9	88.2
Heavy Infection--South Drake									
A-Horizon	729.1	1468.6	452.9	2736.4	463.8	42345	26.3	85.3	109.6
B-Horizon	757.0	1262.1	415.5	2759.2	506.1	48199	26.5	79.1	102.3

Table 2.4. Initial soil exchangeable nutrient levels.

	pH	P ppm	K ppm	Ca meq/100g	Mg meq/100g	Na meq/100g	N %	NH_4-N ppm	NO_3-N ppm
Light Infection--Charlie Olson									
A-Horizon	4.8	2.8	448.5	5.0	2.3	0.08	0.38	8.1	1.2
B-Horizon	5.4	2.8	390.0	3.2	2.1	0.06	0.09	5.6	0.9
Moderate Infection--Bushy Peterson									
A-Horizon	5.0	4.7	241.8	2.2	1.4	0.10	0.19	5.0	1.0
B-Horizon	5.1	2.6	265.2	1.2	0.9	0.08	0.12	4.0	0.7
Heavy Infection--South Drake									
A-Horizon	4.8	1.7	429.0	0.8	1.2	0.2	0.4	10.0	3.3
B-Horizon	4.6	1.5	237.9	0.8	1.2	0.2	0.4	7.5	2.9

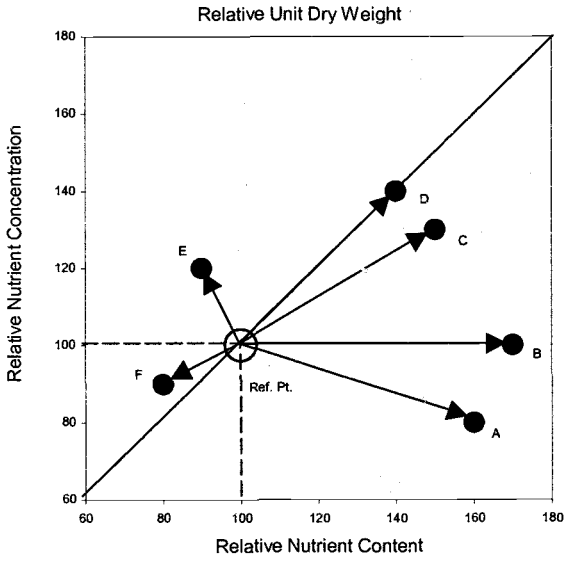
	Fe ppm	Mn ppm	Cu ppm	Zn ppm	SO_4-S ppm	CEC meq/100g	Incub. N ppm	C %	S %	B ppm
Light Infection--Charlie Olson										
A-Horizon	58.0	33.0	0.18	0.70	9.0	48.0	59.6	10.7	<0.01	0.5
B-Horizon	1.4	3.9	0.02	0.02	51.5	25.2	6.0	1.3	<0.01	0.2
Moderate Infection--Bushy Peterson										
A-Horizon	12.0	11.0	0.10	0.16	9.6	26.1	20.8	3.0	<0.01	0.3
B-Horizon	2.0	4.3	0.06	0.06	43.4	25.4	5.4	1.6	<0.01	0.2
Heavy Infection--South Drake										
A-Horizon	5.8	10.0	0.04	0.24	15.5	53.3	32.2	6.6	<0.01	0.5
B-Horizon	7.4	9.6	0.04	0.34	18.1	52.8	16.4	6.0	<0.01	0.4

During December of 2000, three sub-samples of the current year's foliage were collected from six trees from the top one-third of the crown from each treatment replication for foliar nutrient analyses. All foliage samples were dried for 72 hours at 70° C. Needles were removed from the pooled sample and the weight of 100 needles determined for each treatment replication. Dry weight was measured on a sample of 100-needles/pooled sample for use in calculating nutrient contents and vector analysis (Haase and Rose, 1995). Foliage was then ground in a Wiley mill grinder (40 mesh screen). Nutrient concentrations (USAg Analytical Services, Inc., Pasco, WA) were determined from foliage samples using standard laboratory procedures. Relative nutrient concentration, content, and dry weight were calculated (relative to the control treatments) and vector diagrams constructed to facilitate a thorough examination of nutrient responses to the respective treatments.

2.4.4 Vector diagrams

A sample of 100 needles from each treatment replicate was weighed to obtain a unit dry weight for determination of nutrient contents and facilitation of vector analysis (Haase and Rose, 1995). The incorporation of a growth parameter with nutrient concentrations and contents into a vector diagram enables rapid evaluation of treatment effects (Timmer and Stone, 1978; Haase and Rose, 1995). A reference point is chosen (the control treatment in this study) and set equal to 100. Subsequent data points are thus normalized to the reference point. Interpretations

of the significance of the nutrient shift (dilution, sufficiency, deficiency, luxury consumption) can be made based on the relative changes in nutrient content, concentration, and unit dry weight (i.e. the direction and magnitude of the vector) (Figure 2.2, adapted from Timmer and Stone, 1978). The vector diagrams in this study compare nutrient shifts relative to one reference point (the control treatment). Vector diagrams were constructed for the 2000 foliar nutrient samples.



Interpretation/possible diagnosis

A: Dilution (non-limiting)
B: Sufficiency (non-limiting)
C: Deficiency (limiting)
D: Luxury consumption (non-toxic)
E: Excess (toxic)
F: Excess (antagonistic)

Figure 2.2. Interpretation of directional shifts in nutrient concentration, content, and dry weight. Adapted from Timmer and Stone (1978).

2.4.5 Vegetation survey

A vegetation survey was conducted in July 2000 in order to ensure that the complete vegetation removal treatments had good efficacy over all sites. Each individual plot was divided into four equal quadrants. Within each quadrant a circular plot with a five-foot radius was used to determine species percentage presence. Individual plots were pooled in order to establish a plot average.

A stratified rather than random vegetation survey was conducted because many times vegetative species clump together. It is therefore more accurate to spread plots in a grid-like pattern in order to gain a better understanding of the overall population.

2.4.6 Statistical analysis

Using factorial models (Tables 2.5 and 2.6), data were analyzed independently by site using analysis of variance (ANOVA) to determine differences in growth (height and diameter at breast height), which could be attributed to fertilization and/or vegetation control.

Table 2.5. Analysis model used to assess tree height and diameter growth response to fertilization and vegetation control treatments at the South Drake and Charlie Olson study sites.

Source	DF
Block	4
Fertilizer	2
Veg. Control	1
Fertilizer x Control	2
Error	20
TOTAL	**29**

Table 2.6. Analysis model used to assess tree height and diameter growth response to fertilization and vegetation control treatments at the Bushy Peterson study site.

Source	DF
Block	3
Fertilizer	2
Veg. Control	1
Fertilizer x Control	2
Error	15
TOTAL	**23**

Restrictive mean comparisons were made using the Tukey-Kramer method to detect significant differences in data for all effects at the $\alpha \leq 0.05$ level (Ramsey and Schafer, 1997). HDR's were then individually calculated for each tree by dividing the tree height by the DBH. These data were subsequently analyzed using ANOVA. Nutrient and dry weight data were analyzed on each sampling date using

ANOVA. At each sampling date, the Tukey-Kramer procedure was utilized to detect significant differences for all effects at the $\alpha \leq 0.05$ level (Steel and Torrie, 1980). PCR and needle retention analyses were accomplished using ANOVA and Tukey-Kramer's mean comparison. Tests for normality, linearity, and constant variance were performed. No transformations were needed for the data. All analyses were carried out using Statistical Analysis Software V. 8.0 (Statistical Analysis Software Institute Inc., 1999).

2.5 Results

2.5.1 Morphology

On all sites, complete vegetation removal (spray) had a significant positive effect on mean 2000 DBH growth (p-values<.0001), when compared with the non-removal treatment (check) (Figure 2.3). The Charlie Olson site had an increased annual DBH growth of 16%, while both the Bushy Peterson and South Drake sites had annual increases of 19%. Height growth was not significantly different between the two respective vegetation treatments (Figure 2.4). Additionally, no fertilizer treatment had a significant effect on either mean DBH or height growth.

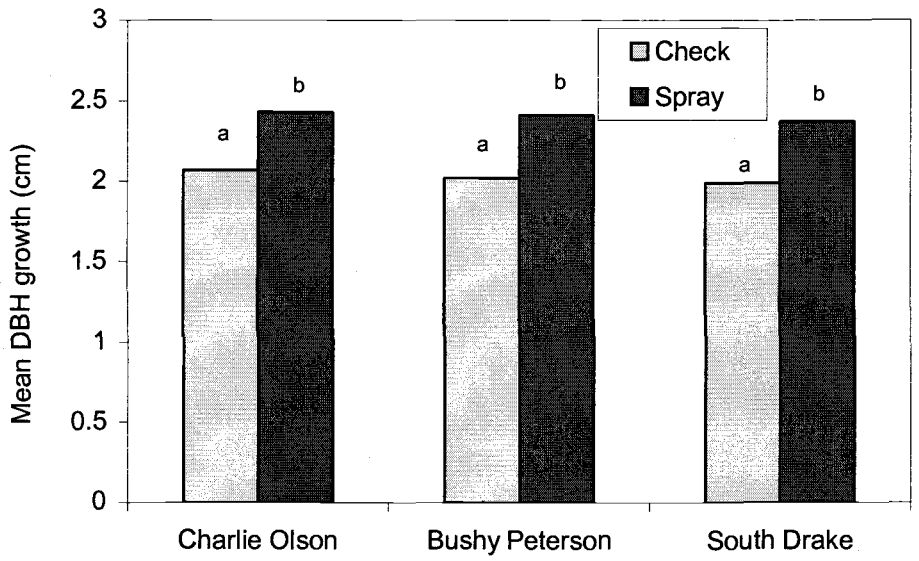


Figure 2.3. Mean 2000 DBH growth (cm). Bars associated with a different letter from the same site are significantly different at the $\alpha \leq 0.05$ level. All sites were analyzed independently of each other.

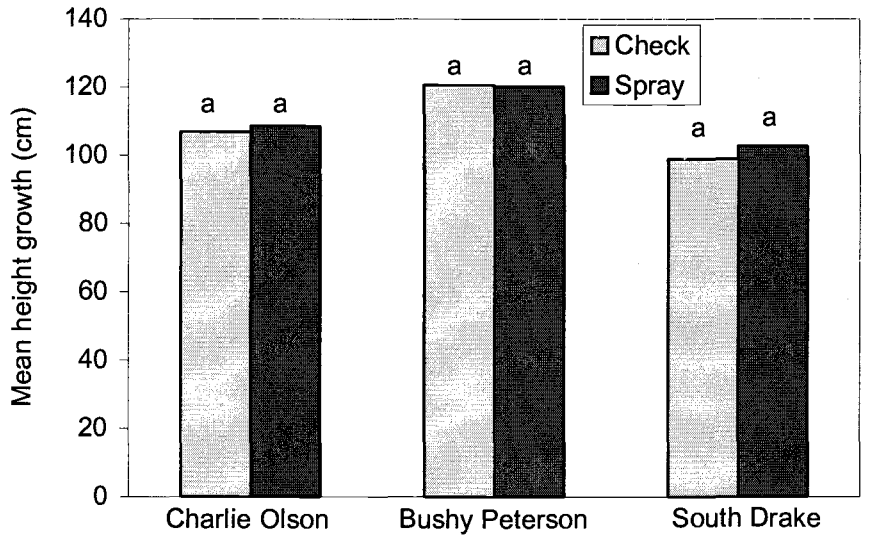


Figure 2.4. Mean 2000 height growth (cm). Bars associated with the same letter from the same site are not significantly different at the $\alpha \leq 0.05$ level. All sites were analyzed independently of each other.

Height to diameter ratios were significantly different between the complete vegetation removal treatment and the non-removal treatment at each respective site after two full growing seasons (Figure 2.5). The vegetation removal treatment lowered HDR's at all sites. At the Charlie Olson site the ratio was separated by 7.5% between treatments (p-value=.0293). The Bushy Peterson site exhibited ratios that differed by 5.7% (p-value=0.0004). The South Drake site was similar to the other two sites with a decreased ratio due to vegetation removal, and a 5.4% difference between the two respective treatments (p-value=0.0056). The fertilizer treatments had no significant impact on height to diameter ratios.

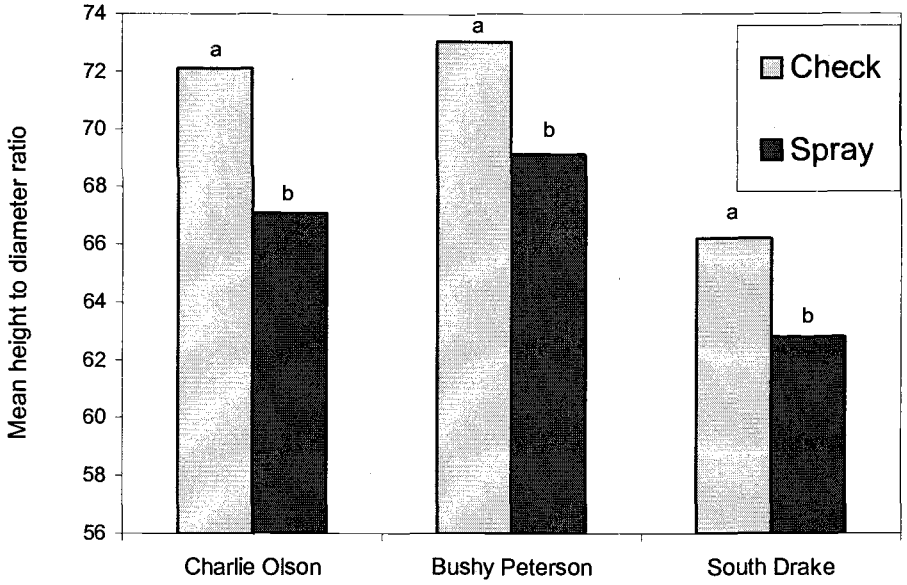


Figure 2.5. Mean 2000 height to diameter ratios. Bars associated with a different letter from the same site are significantly different at the $\alpha \leq 0.05$ level. All sites were analyzed independently of each other.

2.5.2 PG infection (PCR analyses)

There was a significant interaction between fertilizer and vegetation control at the Bushy Peterson site with regard to *PG* infection (p-value=0.0077). A post hoc comparison of treatments at the Bushy Peterson site based on a restrictive means comparison (Tukey-Kramer) resulted in no significant differences between treatments (p-values>0.05). No significant treatment differences were found at the other two respective sites (Figures 2.6, 2.7, and 2.8).

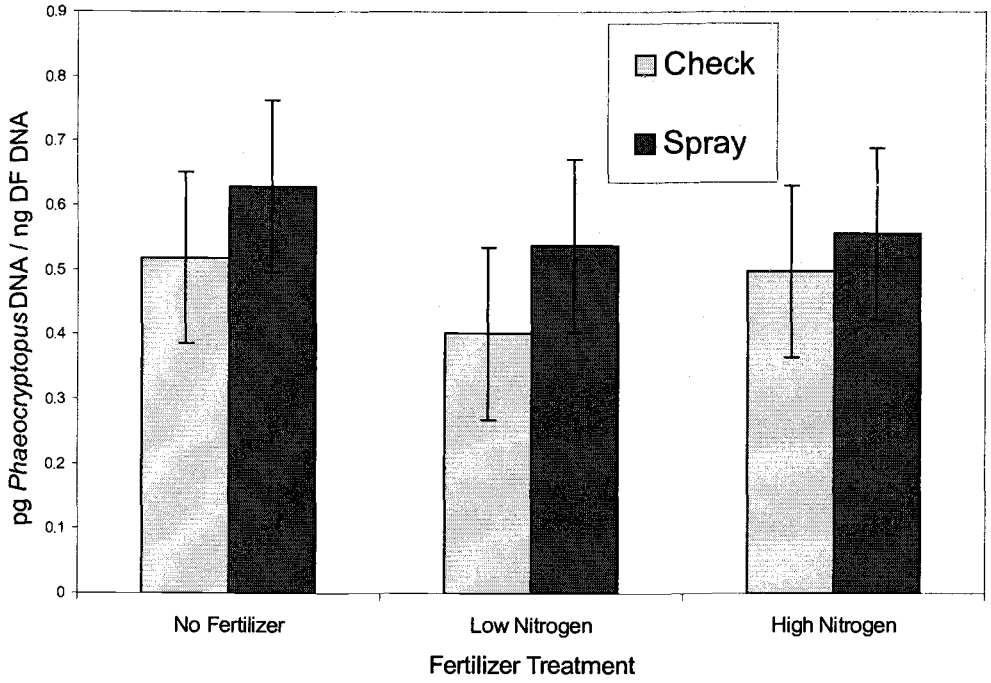


Figure 2.6. 2000 Polymerase Chain Reaction (PCR) results (pg *Phaeocryptopus* DNA/ng Douglas-fir DNA) for the 2000 needle cohort at the Charlie Olson site. Bars are the standard error of the mean.

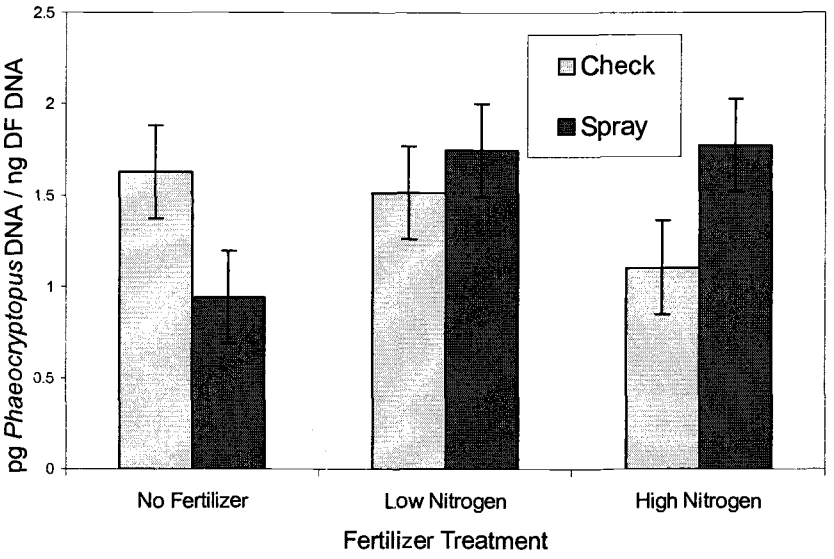


Figure 2.7. 2000 Polymerase Chain Reaction (PCR) results (pg *Phaeocryptopus* DNA/ng Douglas-fir DNA) for the 2000 needle cohort at the Bushy Peterson site. Bars are the standard error of the mean.

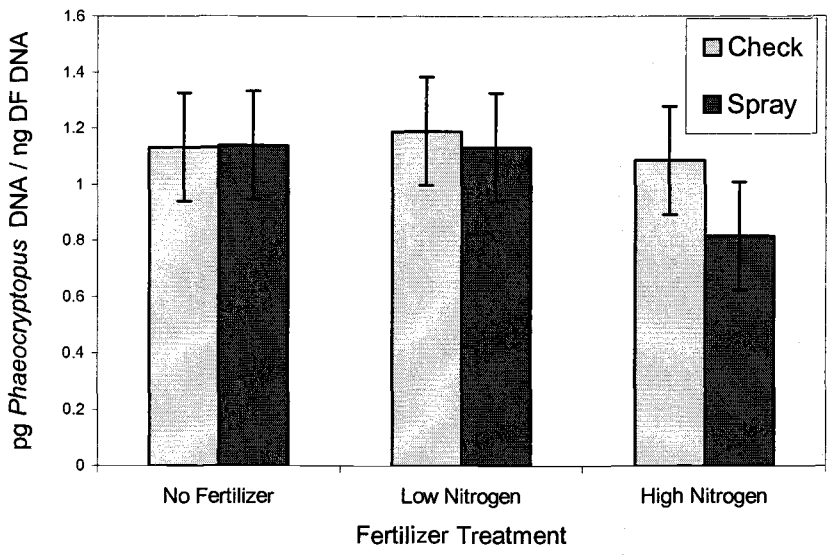


Figure 2.8. 2000 Polymerase Chain Reaction (PCR) results (pg *Phaeocryptopus* DNA/ng Douglas-fir DNA) for the 2000 needle cohort at the South Drake site. Bars are the standard error of the mean.

2.5.3 Needle retention

The 2001 needle retention assessment showed that there was no consistent needle retention or casting response over all sites. However, the most heavily infected site, Bushy Peterson exhibited significantly higher needle retention on the main 1998 (p-value=0.0061), lateral 1999 (p-value=0.0434), and lateral 1998 (p-value=0.0343) cohorts when complete vegetation removal was implemented (Figure 2.9). The Charlie Olson site had significantly increased retention on the 1998 laterals (p-value=.0424), but was non-significant on all other cohorts. The South Drake site was non-significant among all treatments and needle cohorts.

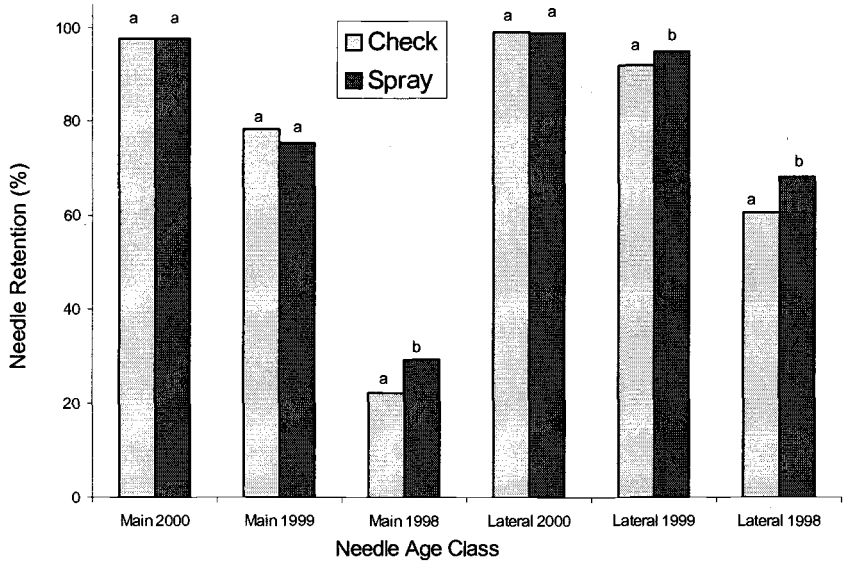


Figure 2.9. Bushy Peterson 2001 needle assessment. Bars associated with a different letter from the same needle cohort are significantly different at the $\alpha \leq 0.05$ level. All branches and cohorts were analyzed independently of each other.

2.5.4 Foliar nutrients

At all sites there was no consistent foliar nutrient concentration response to fertilization other than for boron (Table 2.7). At the Charlie Olson site sulfur (p-value=0.0422) was significantly increased due to fertilizer, as was boron (p-value=0.0471). Potassium (p-value=0.0342), boron (p-value=0.0089), and manganese (p-value=0.0360) were all significantly increased due to fertilization at the Bushy Peterson site. At the South Drake site only iron (p-value=0.0341) and boron (p-value<0.0001) were significantly increased due to fertilization.

Table 2.7. 2001 foliar nutrient p-values associated with respective treatments. Bold values are significant at the $p \leq 0.05$.

Source of Variation[a]	N%	P%	K%	S%	Ca%	Mg%	Na%	B ppm	Zn ppm	Mn ppm	Fe ppm	Cu ppm
Charlie Olson												
$P>F_{2,20}$ (fert)	0.8769	0.8055	0.5644	**0.0422**	0.4931	0.7051	0.4034	**0.0471**	0.4071	0.1397	0.9473	0.1661
$P>F_{1,20}$ (vegcont)	**0.0016**	0.3748	0.2985	1	0.9546	0.5577	0.3525	**0.0037**	0.0645	0.0523	0.0962	0.3933
$P>F_{2,20}$ (fert x vegcont)	0.6111	0.3141	0.1009	0.1407	0.4122	0.5468	0.9813	0.1174	0.4701	0.0671	0.6336	0.1215
Bushy Peterson												
$P>F_{2,15}$ (fert)	0.9376	0.149	**0.0342**	0.9578	0.5648	0.789	0.3196	**0.0089**	0.8936	**0.036**	0.3792	0.3725
$P>F_{1,15}$ (vegcont)	0.1376	0.0834	0.8905	0.8381	0.0509	0.7159	0.5652	0.3273	0.1407	0.5498	**0.0462**	0.1777
$P>F_{2,15}$ (fert x vegcont)	0.2640	0.2186	0.8728	0.4587	0.3009	0.6476	0.8609	0.2886	0.2297	0.2451	0.8080	0.1491
South Drake												
$P>F_{2,20}$ (fert)	0.9919	0.1690	0.7229	0.9426	0.8073	0.7048	0.7139	**<0.0001**	0.9988	0.6148	**0.0341**	0.6344
$P>F_{1,20}$ (vegcont)	0.1518	0.7156	0.5641	0.6504	0.4222	1	0.4933	**0.0023**	0.4537	0.4242	0.4671	0.0891
$P>F_{2,20}$ (fert x vegcont)	0.5495	0.6478	0.1156	0.7346	0.6616	0.3624	0.5067	0.9149	0.3382	0.2440	0.0614	0.6344

[a]fert, fertilizer treatments; vegcont, vegetation control treatments; fert x vegcont, fertilizer and vegetation control interactions.

At the Charlie Olson site there were significant differences in foliar nitrogen between the two vegetation treatments (p-value=0.0016) (Figure 2.10). An 8.4% increase in foliar nitrogen was recognized in the complete removal treatment when compared to the non-removal treatment. The other two sites did not exhibit significant differences in foliar nitrogen (Bushy Peterson; p-value=.1376 and South Drake; p-value=.1518), but they did demonstrate a similar trend to the Charlie Olson site in that their foliar nitrogen levels were increased by 4.5% and 4.3%, respectively. Iron was the only other foliar nutrient that was significantly increased with respect to vegetation control (p-value=0.0462), and that was only at the Bushy Peterson site.

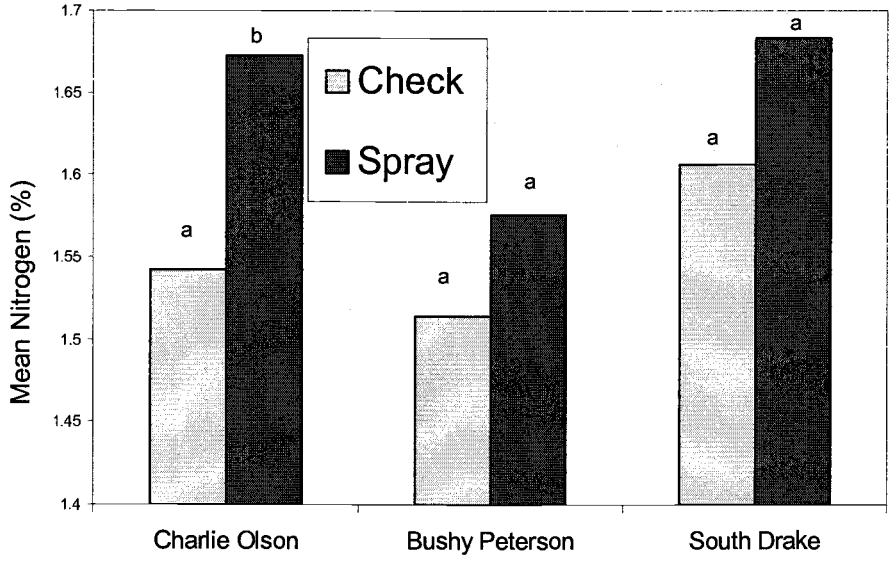


Figure 2.10. Mean 2000 foliar nitrogen concentration (%). Bars associated with a different letter from the same site are significantly different at the $\alpha \leq 0.05$ level. All sites were analyzed independently of each other.

Regression analyses were used to determine if a relationship existed between foliar nitrogen concentration (%) and the amount of *PG* within a needle (based on PCR measurements). At all three respective sites, no significant relationship was established (Charlie Olson, p-value=.9104; Bushy Peterson, p-value=.3476; and South Drake, p-value=.7174). Figure 2.11 represents all measured foliar nitrogen (%) and PCR measurements at the Charlie Olson site. Despite increased foliar nitrogen levels (+8.4%) in the vegetation removal plots when compared to the non-removal plots, there was no significant increase in *PG*.

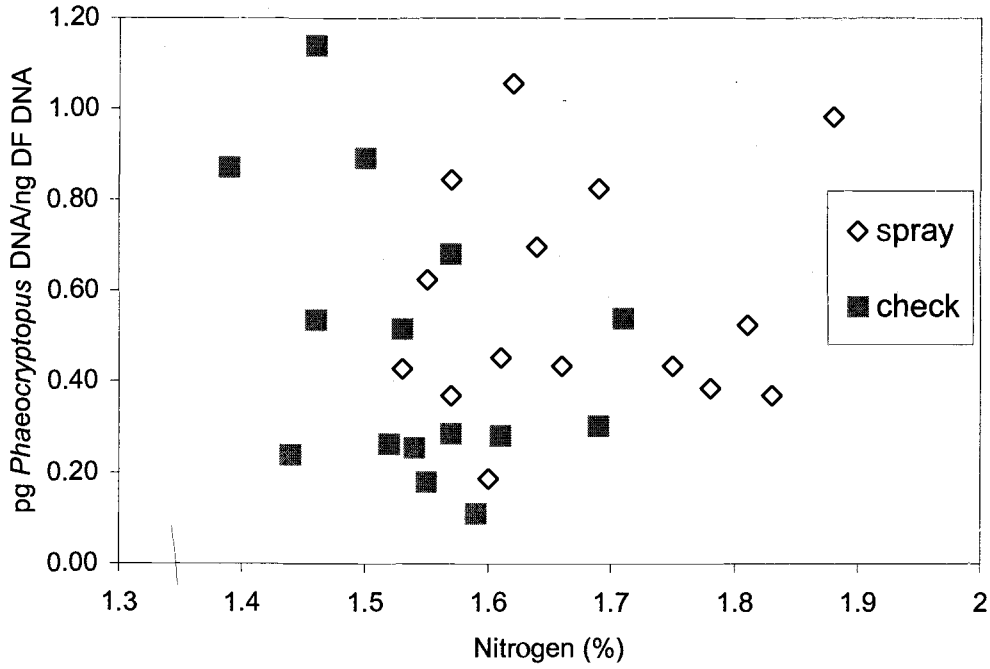


Figure 2.11. 2000 foliar nitrogen concentration (%) levels compared to *PG* infection at the Charlie Olson site. Each data point represents the plot mean.

2.5.5 Vector diagrams

Vector diagrams constructed on the 2000 foliar age class showed a similar distinct pattern with nitrogen over all study sites (Figures 2.12, 2.13, and 2.14). At the Charlie Olson and Bushy Peterson sites, the relative foliar nitrogen contents increased with vegetation removal treatments by approximately 25%. At the South Drake site there was a more moderate increase in foliar nitrogen content of 15%.

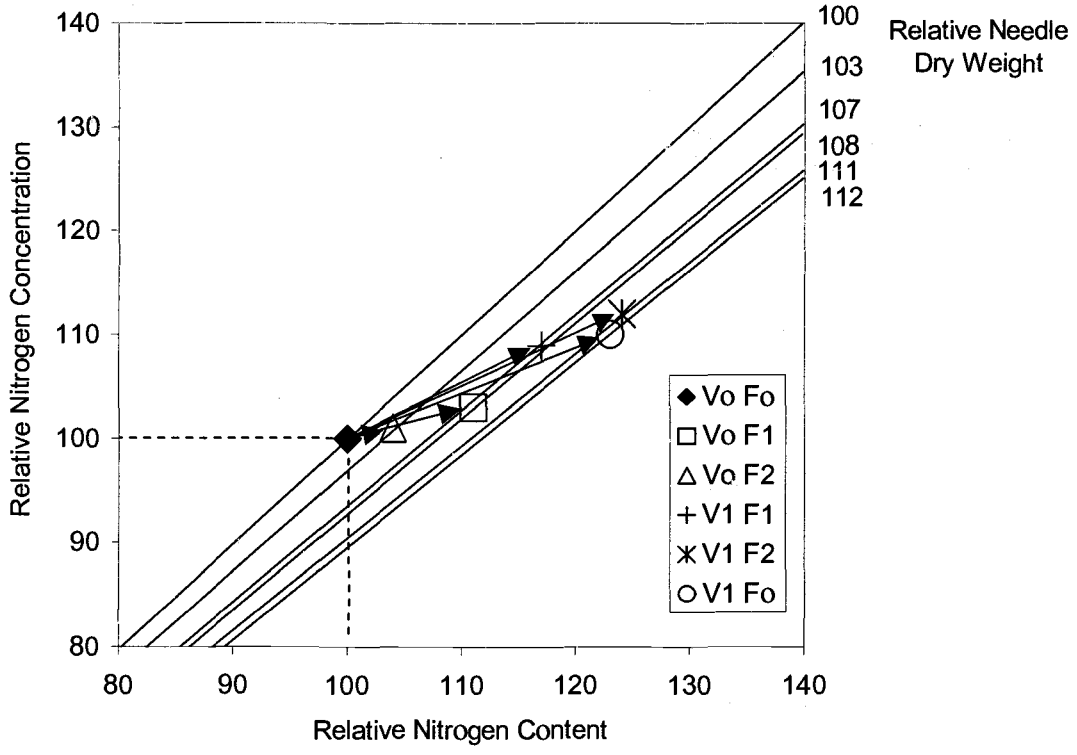


Figure 2.12. Relative nitrogen shift 16 months after initial treatments at the Charlie Olson site. Nitrogen levels were significantly different from the control treatment ($p \leq 0.05$).

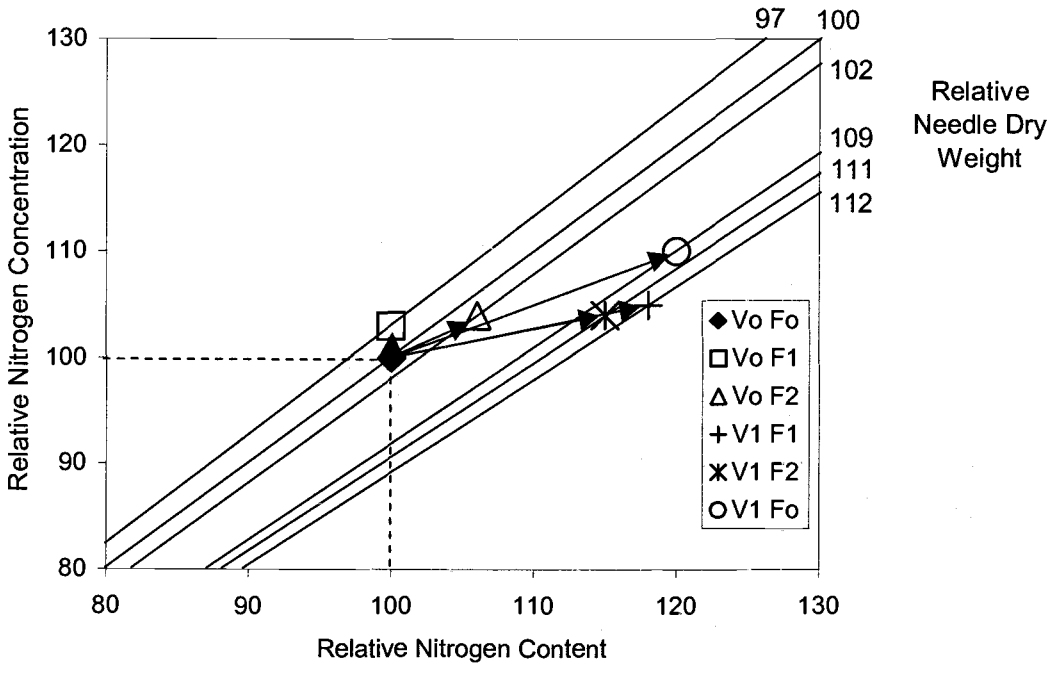


Figure 2.13. Relative nitrogen shift 16 months after initial treatments at the Bushy Peterson site. Nitrogen levels were not significantly different from the control treatment ($p>0.05$).

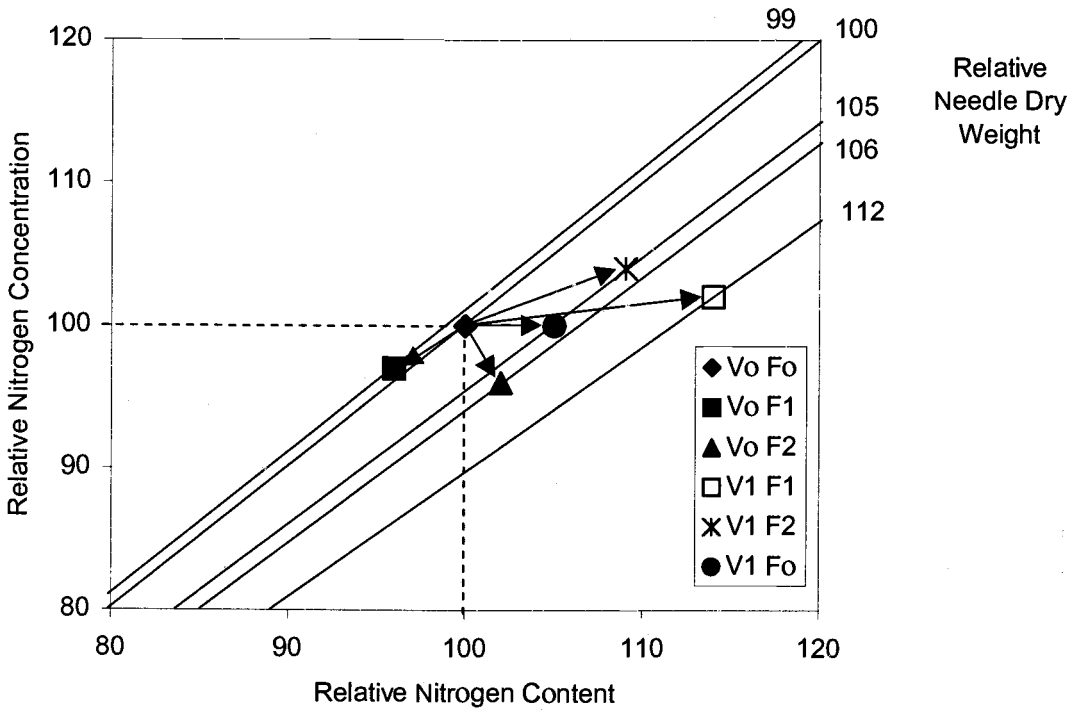


Figure 2.14. Relative nitrogen shift 16 months after initial treatments at the South Drake site. Nitrogen levels were not significantly different from the control treatment ($p>0.05$).

Relative foliar nitrogen concentration was increased slightly by vegetation removal at the Charlie Olson site (10%). No meaningful concentration increases were observed at the Bushy Peterson or South Drake sites. Fertilization treatments showed no increases in either relative concentrations or contents. Relative needle dry weights increased over all treatments at each respective site when compared to the control treatment. The vegetation removal treatment generally had the greatest relative weights.

Of the remaining nutrients tested, boron, zinc, and copper showed similar vector patterns over all sites (data not shown). In general, the relative foliar content of these nutrients was increased 20-25% when compared to the control treatment. The vegetation removal treatment generally had slightly increased foliar content levels when compared to the fertilizer with no vegetation removal. All other nutrients that had vector diagrams constructed showed no distinct patterns among treatments (phosphorus, potassium, calcium, magnesium, and sulfur) (data not shown).

2.5.6 Sapling needle weights

At the three respective sites, needle weights were the greatest in plots where vegetation removal was implemented. There were significant treatment differences between the vegetation removal treatment and the non-removal treatment with regard to mean weight per 100 oven-dried needles for the 2000 cohort needles on the Charlie Olson (p-value=0.0243) and Bushy Peterson (p-value=0.0038) sites (Table 2.8). The South Drake site did not demonstrate significant differences among any treatments (p-value≥0.05). However, there were distinct trends between the vegetation removal treatment and the non-removal treatment at the South Drake site.

Table 2.8. 2000 sapling needle weights (grams). Means within the same column associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses. All sites were analyzed independent of each other.

Site	Treatment	2000 Cohort
Charlie Olson	Check	0.55 (0.01) a
	Spray	0.59 (0.01) b
p>F = 0.0243		
Bushy Peterson	Check	0.58 (0.01) a
	Spray	0.65 (0.01) b
p>F = 0.0038		
South Drake	Check	0.57 (0.01) a
	Spray	0.60 (0.01) a
p>F = 0.1051		

At the Charlie Olson site a 7.3% increase in needle weight was recognized with vegetation removal. The greatest difference in needle weights between vegetation treatments occurred at the Bushy Peterson site, where a 12% increase was observed. The South Drake site was similar to the Charlie Olson site with a respective 5.2% increase. Fertilization played no significant role in needle weight results.

2.5.7 Vegetation survey

Vegetation cover over all sites was reduced by approximately 85% in the vegetation removal plots. In addition, vegetation height was reduced by approximately 75%. Virtually all vegetation that remained consisted of herbaceous species. Shrubs and woody competitors were successfully eliminated from all vegetation removal plots.

2.6 Discussion

The removal of competing vegetation on all sites did not result in significant treatment responses after the first growing season (1999). No significant growth differences were detected during the 1999-growing season most likely because the vegetation removal treatment had not been implemented long enough for the trees to benefit from the decreased competition. However, DBH growth significantly increased during the 2000 growing season at all sites in the vegetation removal plots. There was no significant height growth response in either the 1999 or 2000 growing season.

Thinning treatments in young dense coniferous forest stands are known to increase basal area growth but often not height growth (Alexander, 1960; Weetman, 1971). This likely explains why the vegetation removal treatment at present has had no significant effect on height growth.

No fertilizer treatment had any significant effects on height or DBH growth in either respective growing season. There could be several reasons why fertilization has not significantly affected either height or DBH growth. The first reason, and most suspect, is that there was little initial fertilizer uptake by the trees. Due to dry conditions during and after fertilization, the six-month time-released blend that was applied may never have been utilized. The 2000 growing season had 28% less rainfall than average during the month of March through the end of August near the South Drake site (NWS weighing rain gage, HMSC, Newport, OR USA). Similar drought conditions occurred at the other two respective sites.

Foliar nutrient analyses support this conclusion, because there were no consistent foliar nutrient concentration responses to fertilization other than for boron. Although not significant, there was an increasing trend in foliar nitrogen (4.3%, 4.5%, and 8.4%) at all sites with complete vegetation removal. Piene (1978) found that decreasing competition in young stands resulted in greater foliar nitrogen concentrations. Additionally, the optimization between the variable nutrient requirements of these saplings is complicated by the variable availability of nutrients on forest sites during crop development (Mahendrappa *et al.*, 1986). It may also be that nutrients were not a limiting growth factor at this point in time.

The height to diameter ratios were significantly different for vegetation treatments but not for fertilizer treatments. The same pattern recognized for DBH growth in 2000 was recognized for the height to diameter ratios. This makes sense because if the DBH of one tree is increased and another tree has a smaller DBH

with both heights being equal, then the tree with the larger DBH would have a lower HDR. Additionally, research suggests that generally higher HDR's are obtained where the percentage cover of vegetation is relatively high and lower HDR's are obtained where the percentage cover of vegetation is relatively low (Opio *et al.*, 2000).

Based on research from spacing and thinning treatments in young dense northern coniferous forests, increases in nitrogen and other nutrients generally occur in the 2nd and 3rd year after treatment (Weetman, 1971; Piene, 1978). This same trend may be developing in this study. Thus, there is potential for future increases in nitrogen and other nutrients due to vegetation removal. Therefore, it is not presumptuous to think that growth responses will continue.

After two full growing seasons, the Bushy Peterson site was the only site to exhibit a significant difference among treatments related to *PG* infection. Specifically, a significant interaction between fertilizer and vegetation removal was recognized at the Bushy Peterson site. However, as reported previously, there were no significant differences between treatments after a restrictive means comparison test.

It is interesting to note that based on PCR analyses, the Bushy Peterson site was more heavily infected on average than the South Drake site. This is especially interesting for two distinct reasons. First, the Bushy Peterson site is located approximately 25 miles inland from the coast, whereas the South Drake site is within five miles of the coast with each nearly lying on the same latitudinal line. It

is commonly thought that the highest *PG* infection levels are within 15 miles of the coast. Secondly, despite having an infection level that was 34% less than the Bushy Peterson site, the South Drake site had lower overall needle retention (Figure 2.15). It is possible that the most infected needles at the South Drake site were cast and those that remained carried less infection. Therefore, the infection differences among sites may be a direct result of needle casting. The increased infection at the Bushy Peterson site could potentially be why an interaction effect was revealed. Past research suggests that opening young plantations up by pre-commercial thinning and/or intense vegetation removal has no impact on the severity of SNC as measured by pseudothecia presence (Hood and Sandberg, 1979).

Speculation that an increase in the relative availability of nitrogen can lead to increased susceptibility to *PG* in the Oregon Coast Range has been suggested recently (Waring *et al.*, 2000). Based on data from the Charlie Olson site where nitrogen concentration levels were increased by 8.4% through vegetation removal, no significant increase in *PG* infection was measured in this study. Thus, the hypothesis that a single relationship exists between the concentration of nitrogen in foliage and increased damage from SNC is not supported.

Needle retention on trees at the Bushy Peterson site increased due to vegetation removal treatments on the main 1998, lateral 1999, and lateral 1998 needle cohorts. The Charlie Olson site had increased retention on the 1998 laterals only, and there was no response at the South Drake site. Hood and Sandberg

(1979) found in New Zealand that five years after severely infected 17-year old stands were thinned to 90 and 300 stems per acre, both needle retention and needle density was equivalent to that in unthinned plots with 1200 stems per acre. Contradictory to this research, some visual observations suggest that increased openness in a stand through intense vegetation removal or thinning may actually decrease overall needle retention.

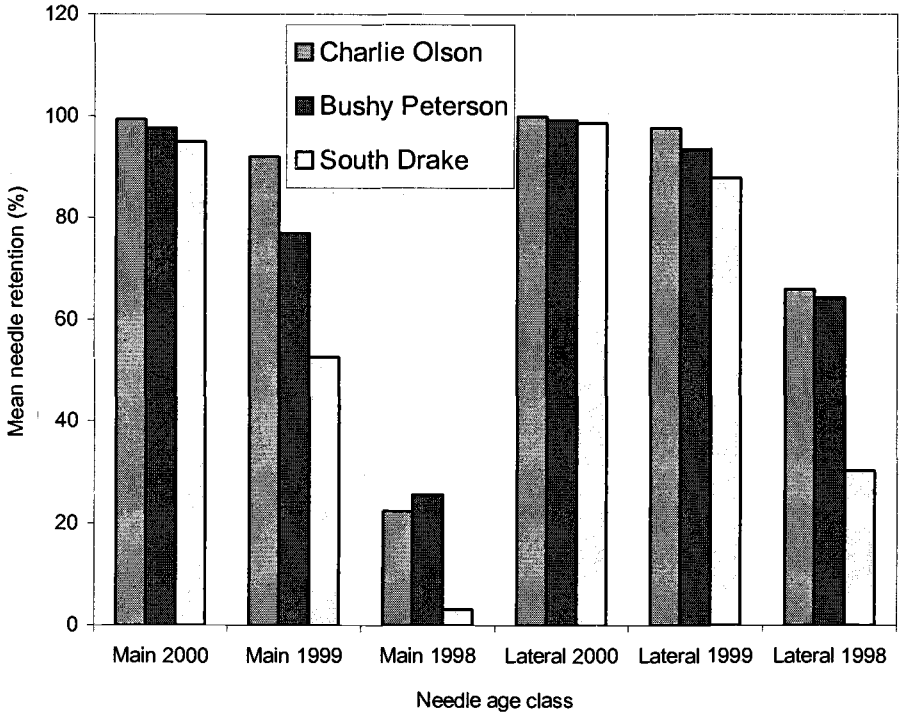


Figure 2.15. 2001 needle retention assessment by site.

2.7 Conclusion

Current research indicates that vegetation removal in sapling Douglas-fir stands is beneficial. A positive growth response (increased DBH growth) and no increased *PG* infection within treatments make vegetation removal and control a potentially useful management tool in young Douglas-fir plantations. However, in operational settings it may be difficult to obtain the same levels of vegetation control as exhibited in this study due to intense removal procedures (manual and herbicide) that may not be practical or economically feasible.

With more time it will be possible to determine the lasting effects that vegetation control has with regard to current documented growth responses. Research continues to be ongoing with fertilizers, specifically with a focus on potential growth responses and nutrient effects on fungal colonization.

2.8 Acknowledgements

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for information and convenience of the reader and does not constitute official endorsement or approval by Oregon State University.

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Chapter 3

Effect of Elemental Sulfur on Swiss Needle Cast and Growth of Coastal Douglas-fir Saplings

by

Gabriel A. Crane

3.0 Abstract

The objective of this experiment was to determine how elemental sulfur (Thiolux®), and Bravo® (chlorothalonil) fungicide affect Swiss needle cast (SNC) caused by *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*), and subsequent growth in Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) saplings. Concurrently, foliar and soil nutrient change and needle retention were of interest. Sulfur was applied as a foliar application with and without TacTic® sticker on individual trees in an existing six-year-old Douglas-fir plantation in the Oregon Coast Range five miles north of Toledo, OR. Additionally, a sulfur ground and foliar Bravo® fungicide application were applied. Bravo® fungicide applied at a rate of 3.75 pts/100 gal of water sprayed on the foliage for 14 seconds resulted in a significant reduction in infection when compared to all other treatments. The sulfur with-sticker was also significantly effective at lowering *PG* levels when compared to the control treatment, but 10 times less effective than the Bravo® treatment. All other treatments were not significant in lowering *PG* levels when compared to the control treatment. Trees that received the Bravo® fungicide treatment had significantly greater foliar nitrogen levels (+20%) nine months after treatment application when compared to the control treatment. However, the foliar nitrogen levels in the Bravo® treated saplings did not differ significantly from the control treatment after 18 months. Foliar treatment applications of both sulfur with (+86%) and without sticker (+57%) led to significantly increased levels of foliar sulfur.

Height and diameter at breast height (DBH) were not significantly affected by any treatment after two growing seasons. Needle retention was not significantly different among treatments after the initial growing season.

3.1 Introduction

The infection and colonization of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) needles by the ascomycete *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*), results in a foliar disease known as Swiss needle cast (SNC). The disease is caused by internal needle colonization and subsequent stomatal blockage by ascomata of *PG* (Winton, 2001). The fungus utilizes products of photosynthesis, thus reducing assimilation rates of the foliage and impairing needle function. Diseased trees develop symptoms of needle chlorosis, decreased needle retention, and potential losses in overall height and diameter growth. Severe disease symptoms are associated with extensive colonization of foliage less than one-year old (Hansen *et al.*, 2000).

The use of elemental sulfur as a fungicide is known to extend back for at least a century, when limited use of the material was made in France in combating grape mildew (Groves, 1942). Today, elemental sulfur in a small particle size is widely employed in agriculture as a protective fungicide and/or fertilizer. The material used is commonly a dry wettable form applied as a liquid spray. The increase in effectiveness of elemental sulfur fungicides over the last several

decades results from smaller particle sizes that in turn relates to better coverage and adhesion of the finer materials. This in turn results in increased surface exposure of the sulfur particles.

It is understood that a very definite relationship exists between particle size and the effectiveness of a sulfur fungicide (Groves, 1942). Understanding the mode of action of sulfur in affecting the destruction of fungal life is important when developing treatment options. Additionally, it is of importance to recognize differing levels of efficacy associated with the fineness of sulfur material. Much attention has been devoted to the problem, and it is generally accepted that sulfur reaches and penetrates into a spore or other fungous structure on sprayed fruit or foliage through the vaporization or escape of sulfur molecules directly from the particles (Groves, 1942). Therefore, a sulfur vapor is maintained and reaches any fungal tissue nearby and causes its destruction through complex vital processes wherein the fungus literally destroys itself through the production of highly toxic hydrogen sulfide (Groves, 1942; Tweedy, 1981).

Based on field surveys and glasshouse seedling experiments, the sulfur status of trees and the relationship between sulfur and nitrogen nutrition have been suggested as one critical group of factors affecting tree health (Lambert and Turner, 1977). The physiological role of sulfur is closely associated with that of nitrogen and, as a component of the amino acids cysteine, cystine, and methionine, sulfur is vital to protein synthesis (Lambert, 1986). Lambert and Turner (1977) hypothesized that sulfur deficiency, possibly induced by high nitrogen levels (as is

common in the Oregon Coast Range), leads to high foliar arginine accumulations and that these can be utilized by fungal pathogens, such as *PG*, thus causing rapid infection. Lambert *et al.* (1976) demonstrate a direct relationship between plant nutritional status and pathogen infection in an experiment with *P. radiata* seedlings. Thus, evidence exists that biochemical imbalances caused by nutrient deficiency can provide food sources (amino acids) to invasive fungi.

Over the past 10 years there has been an increasing incidence as well as damage due to SNC in the Oregon Coast Range. There is an immediate need to find a remedy to increase the overall growth and vigor of these affected Douglas-fir trees. In 1997, an observational experiment using elemental sulfur suspended in water and sprayed on the trees in an unknown amount increased sapling basal area growth by 18% after two years (Personal communication, Mark Gourley, Starker Forests Inc., 1999). Those results were encouraging enough to warrant further investigation to better understand the potential for elemental sulfur applications to lessen SNC impacts on Douglas-fir saplings. Additionally, this study is focused on infection severity measured through both needle retention and quantitative real-time Polymerase Chain Reaction (PCR) assay developed specifically for quantification of *PG*. Foliar nutrient status, specifically nitrogen and sulfur were monitored to determine possible needle absorption of sulfur from foliar applications or sulfur translocation via the xylem from ground treatments.

3.2 Materials and methods

3.2.1 Study site

The study site was installed near Toledo, Oregon in an existing six-year-old Douglas-fir plantation in May 1999. The trees were moderately to heavily infected with *PG* (needle retention <2 years). The plantation had a stocking density of 310 trees/acre. It is located in T. 10S. R. 10W. Sec. 19. The soils are Tolovana of the Reedsport Association. The site index (50) is 136. The study was implemented on industrial land owned by The Timber Company (currently Plum Creek Timber Co.).

3.2.2 Experimental design and treatments

This experiment utilizes a complete randomized design with 10 replications (trees) for each of the five treatments. Treatments were assigned randomly to trees utilizing the entire site. Selected trees were at least 6 m apart to avoid treatment drift from nearby applications. Trees with forked stems or which originated from natural regeneration were not used for measurement trees. Each tree was clearly identified with an aluminum tag, marking paint, and flagging.

There are five treatments in the study:

1) Untreated control
2) Bravo®(chlorothalonil) fungicide @ 3.75 pts/100 gal sprayed on the foliage

3) Sulfur (Thiolux®) diluted with water (25 lb per 100 gal) sprayed on the foliage
4) Sulfur (Thiolux®) diluted with water (25 lb per 100 gal) with TacTic® sticker (8 oz per 100 gal) sprayed on the foliage
5) Sulfur (Thiolux®) diluted with water (25 lb per 100 gal) sprayed on the ground under each tree within the drip line

Treatments were applied on June 8, June 25, and July 10, 1999 using a truck tank sprayer at 38 psi. Each tree was sprayed evenly top to bottom for 14 seconds resulting in an application rate of 2 oz Thiolux® per tree. This resulted in a "wetting" of the needle surface area. Treatments were applied at two week intervals that corresponded with shoot elongation and the peak period for needle infection, when there is new needle growth and plentiful moisture creating ideal infection conditions. It is important to note that a needle can only become infected during the first three months following elongation. After this, the needle is resistant to fungal colonization (Personal communication, Dr. Jeff Stone, Oregon State University, 2000).

Thiolux® is a dry flowable micronized sulfur. The micronized or small particle formulation is designed to provide more thorough spray coverage. Compared to a granular material, Thiolux® also provides a more readily available supply of sulfur for crop growth. The Thiolux® treatments are referred to as sulfur treatments throughout this paper.

3.2.3 Measurements and sampling

Initial diameter at breast height (DBH) and height were measured in May 1999 (Figure 3.1). Measurements were also recorded in October 1999 and 2000. Height measurements were taken using Crain® 11 m telescoping fiberglass rods. DBH measurements were taken with Spencer® diameter tapes. Seasonal growth is calculated by subtracting the previous years growth from the current years growth. Height to diameter ratio (HDR) is an individual tree based index and is calculated by dividing the height of the measure tree by the DBH of the tree.

An initial SNC assessment was made in mid-July 1999 on a branch at breast height. This same branch was used for continuing assessments of *PG* infection. Assessments are made on a yearly basis during May of each year from the same branch where the previous assessment was taken. The assessment consisted of ocularly estimating the percentage of needle retention for each cohort of needles on the main stem of the branch and also on a lateral marked for future assessments (Figure 3.2).

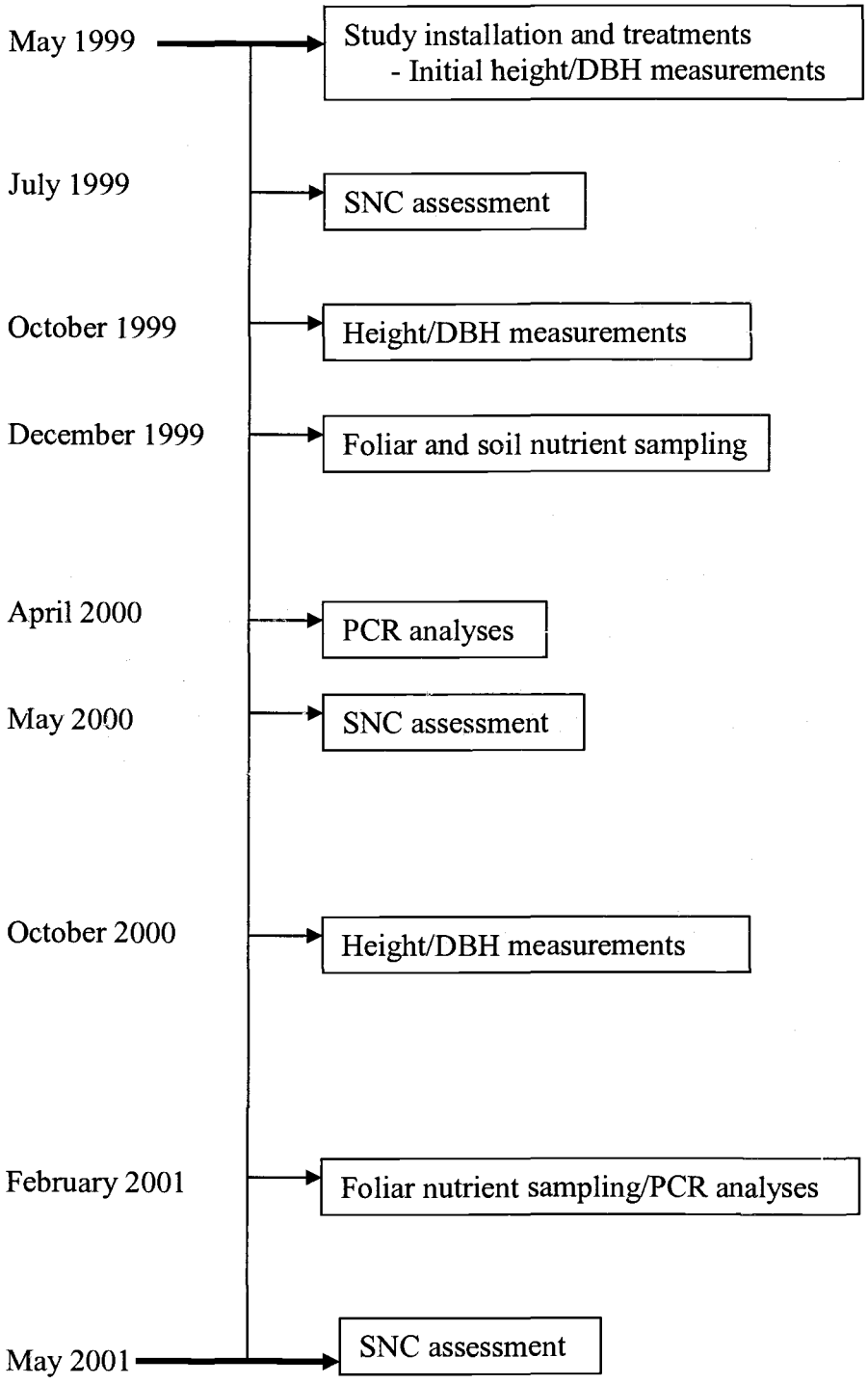


Figure 3.1. Timeline of treatments and measurements.

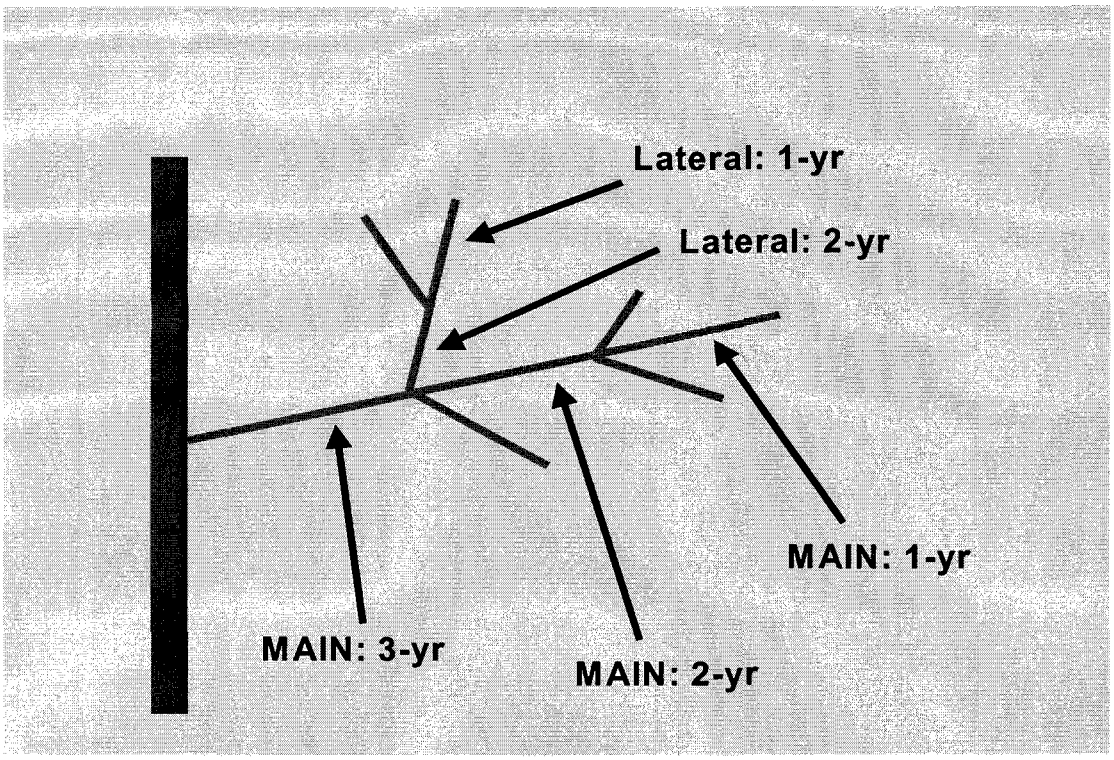


Figure 3.2. Needle cohort retention assessment diagram.

When monitoring the disease on an individual tree level, needle retention has correlated well with disease severity (Filip *et al.*, 2000). By tagging and using the same branches for monitoring purposes every year, it was possible to follow the progression or decline of the disease.

In April 2000, current-year foliage was sampled on all 50 trees. Five samples were taken from the top one-third of the crown. The samples were pooled and the pooled sample used for analysis. Using the PCR procedure, the relative amount of *PG* fungus within needles was identified. PCR results in a ratio of

picograms of *PG* DNA to nanograms of Douglas-fir DNA. PCR was used because it has the advantage of speed, technical simplicity, low detection limits, and specificity over other procedures (Stone *et al.*, 1999). This procedure has the ability to determine the exact level of fungus within an individual needle.

In February 2001, both current-year (2000 cohort) and second-year (1999 cohort) foliage was sampled on all 50 trees in order to evaluate the effectiveness of treatments after one full growing season with respect to infection levels. PCR analyses were implemented on these samples.

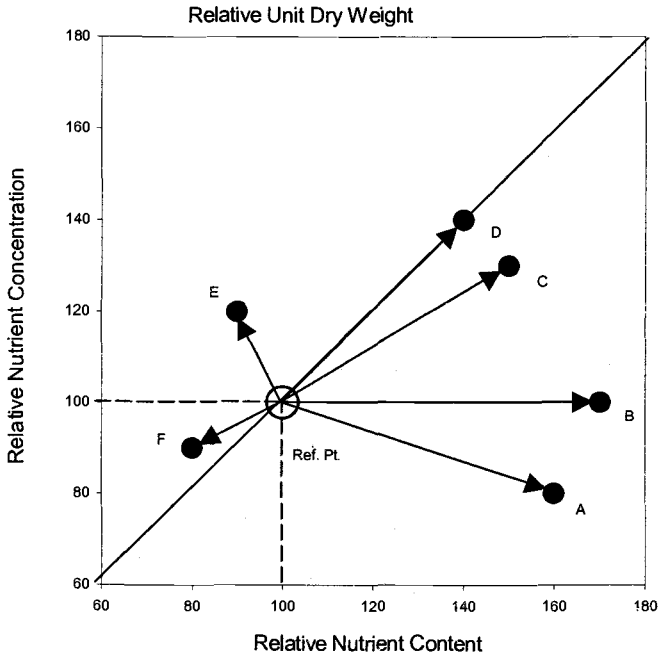
Foliar and soil nutrient samples were collected in December of 1999. Current year foliage was collected from mid-canopy on four randomly selected trees from each treatment. Three sub-samples from each tree were collected and pooled for analyses. Soil was collected from the base of the same four trees at three different locations within the drip line. Soil nutrient concentrations were determined from soil samples using standard laboratory procedures (Oregon State University Central Analytical Laboratory). The soil from the three locations from each tree was mixed to obtain one characteristic sample for each tree. Soil was collected from the first six inches of the A-horizon.

During December of 2000, three sub-samples of the current-year foliage were collected from all treatment trees at mid-canopy. The three sub-samples were pooled by individual tree for analyses.

All foliage samples were dried for 72 hours at 70° C. Needles were removed from the pooled sample and the weight of 100 needles determined for

each individual tree. Dry weight was measured on a sample of 100 needles/pooled sample for use in calculating nutrient contents and vector analysis (Haase and Rose, 1995). Foliage was then ground in a Wiley mill grinder (40-mesh screen). Nutrient concentrations were determined from foliage samples using standard laboratory procedures (USAg Analytical Services, Inc., Pasco, WA). Relative nutrient concentration, content, and dry weight were calculated (relative to the control treatments) and vector diagrams constructed to facilitate a thorough examination of nutrient responses to the respective treatments.

A sample of 100 needles from each treatment replicate was weighed to obtain a unit dry weight for determination of nutrient contents and facilitation of vector analysis (Haase and Rose, 1995). The incorporation of a growth parameter with nutrient concentrations and contents into a vector diagram enables rapid evaluation of treatment effects (Timmer and Stone, 1978; Haase and Rose, 1995). A reference point is chosen (the control treatment in this study) and set equal to 100. Subsequent data points are thus normalized to the reference point. Interpretations of the significance of the nutrient shift (dilution, sufficiency, deficiency, luxury consumption) can be made based on the relative changes in nutrient content, concentration, and unit dry weight (i.e. the direction and magnitude of the vector) (Figure 3.3, adapted from Timmer and Stone, 1978).



Interpretation/possible diagnosis

A: Dilution (non-limiting)
B: Sufficiency (non-limiting)
C: Deficiency (limiting)
D: Luxury consumption (non-toxic)
E: Excess (toxic)
F: Excess (antagonistic)

Figure 3.3. Interpretation of directional shifts in nutrient concentration, content, and dry weight. Adapted from Timmer and Stone (1978).

The vector diagrams in this study compare nutrient shifts relative to one reference point (the control treatment). Vector diagrams were constructed for both 1999 and 2000 foliar nutrient samples.

3.2.4 Statistical analysis

Height and DBH measurements were analyzed using analysis of variance (ANOVA). Mean comparisons were made using the Waller-Duncan method to detect significant differences in data for all effects at the $\alpha \leq 0.05$ level (Ramsey and Schafer, 1997). HDR's were then individually calculated for each tree by dividing the tree height by the DBH. These data were subsequently analyzed using ANOVA. Nutrient and dry weight data were analyzed on each sampling date using ANOVA. At each sampling date, Fisher's Protected Least Significant Difference (FPLSD) procedure was utilized to detect significant differences in data for all effects at the $\alpha \leq 0.05$ level (Steel and Torrie, 1980). PCR and needle retention analyses were accomplished using ANOVA and Waller-Duncan's mean comparison. Tests for normality, linearity, and constant variance were performed. All analyses were carried out using Statistical Analysis Software V. 8.0 (Statistical Analysis Software Institute Inc., 1999).

3.3 Results

3.3.1 General results

Sapling morphological development (height and DBH) was not significantly affected two years after treatment. Additionally, height to diameter ratios were not significantly different among treatments.

Six months after the treatments were applied there was no significant difference in soil nutrients among treatments. Saplings that received the Bravo® fungicide treatment demonstrated the largest increase in foliar nitrogen levels after six months when compared to the control.

Bravo® was the most effective treatment in reducing the colonization of *PG* in treated foliage (1999 cohort). The 2000-needle cohort showed no significant differences among treatments. There were no treatment effects on mean weight per 100 oven dried needles for the 1999 cohort needles sampled during December of 1999 or the 2000 cohort needles sampled in December 2001. There were no significant differences in mean needle retention among treatments for both the May 2000 and May 2001 needle assessments.

3.3.2 Morphology

There were no significant differences among treatments in DBH or height growth after the first growing season (1999). These results were expected, as the respective treatments did not yet have time to integrate treatment effects into significant growth impacts. Two full growing seasons (Fall 2000) after the treatments were implemented there were no significant differences among treatments in DBH or height growth (Table 3.1). Additionally, there were no significant differences in height to diameter ratios between treatments after two growing seasons (Table 3.2).

Table 3.1. Mean 1999 and 2000 height and DBH growth. Treatments (1999) within columns associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

	1999		2000	
Treatment	DBH Growth (cm)	Height Growth (cm)	DBH Growth (cm)	Height Growth (cm)
Control	1.69 (0.09) a	98.1 (8.7) a	2.22 (0.15) a	112.0 (8.6) a
Bravo®	1.80 (0.09) a	98.4 (8.7) a	2.26 (0.15) a	107.5 (8.6) a
Sulfur w/ sticker	1.88 (0.09) a	103.5 (8.7) a	2.34 (0.15) a	103.5 (8.6) a
Sulfur no sticker	1.91 (0.09) a	100.8 (8.7) a	2.20 (0.15) a	101.5 (8.6) a
Sulfur ground	1.64 (0.09) a	102.1 (8.7) a	1.98 (0.15) a	92.0 (8.6) a
P > F	0.1968	0.9904	0.5264	0.5624

Table 3.2. Mean 2000 height to diameter ratios by treatment at the sulfur study. Standard error of the mean is in parentheses.

Treatment	**Height/Diameter Ratio**
Bravo®	62.56 (2.2)
Sulfur w/sticker	63.17 (2.2)
Sulfur no sticker	65.47 (2.2)
Sulfur ground	63.65 (2.2)
Control	67.99 (2.2)
P>F = 0.4139	

3.3.3 Soil nutrients

There was no significant difference (p-values≥0.1) among treatments for any of the soil nutrients tested in December 1999 (Table 3.3). However, the sulfur ground application almost doubled the control treatment when looking specifically at SO_4 ppm. Although not statistically significant due to a small sample size (n=4) and a relatively high variance, there was a definite trend. This demonstrates that there was an increased amount of SO_4 potentially available for uptake.

Table 3.3. 1999 mean soil nutrients. Treatments associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatment	N %	P ppm	K ppm	SO_4 ppm	Ca meq 100g	C %	pH
Control	0.66 (0.06) a	6.75 (0.32) a	405.8 (32.1) a	17.28 (3.5) a	1.3 (0.24) a	11.93 (0.92) a	4.60 (0.07) a
Bravo®	0.54 (0.06) a	5.75 (0.32) a	364.5 (32.1) a	22.28 (3.5) a	0.8 (0.24) a	9.70 (0.92) a	4.73 (0.07) a
Sulfur w/ sticker	0.68 (0.06) a	5.75 (0.32) a	371.3 (32.1) a	27.25 (3.5) a	1.6 (0.24) a	12.80 (0.92) a	4.58 (0.07) a
Sulfur no sticker	0.63 (0.06) a	5.67 (0.36) a	376.7 (35.9) a	20.47 (3.9) a	1.1 (0.27) a	11.20 (1.03) a	4.57 (0.01) a
Sulfur ground	0.59 (0.06) a	5.50 (0.32) a	380.3 (32.1) a	31.40 (3.5) a	0.9 (0.24) a	11.48 (0.92) a	4.58 (0.07) a
P > F	0.645	0.295	0.964	0.265	0.527	0.496	0.676

3.3.4 Foliar nutrients

Trees that received the Bravo® fungicide treatment had significantly greater nitrogen levels in the 1999 foliage than the control treatment (p-value=.0037) six months after treatment (Table 3.4). The foliar sulfur with-sticker application had moderately significant increases in foliar nitrogen when compared to the control treatment (p-value=.01). All other treatments were not significantly different from the control.

The sulfur with-sticker and sulfur with-no-sticker application differed significantly from the control treatment when comparing sulfur nutrient levels six months after treatment (p-values=.0003 and .0043, respectively). All other nutrients tested did not differ significantly from the control treatment.

The 2000 needle cohort nitrogen levels were significantly different in the sulfur with-no-sticker (p-value=.0061) and sulfur ground (p-value=.0332) treatments when compared to the control treatment 19 months after treatment applications (Table 3.5). The Bravo® treatment, which had shown a significant response after six months was not significantly different from the control 19 months after treatment.

Sulfur levels were increased significantly from the control treatment for all treatments except Bravo®, which was only marginally significantly increased (p-value=.0572). Additionally, the Bravo® treatment had significant increases in boron when compared to the control treatment (p-value=.0007). All other nutrients showed no significant increases among treatments.

Table 3.4. 1999 foliar nutrients. Treatments associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatments	N %	P %	K %	S %
Control	1.52 (0.06) a	0.15 (0.01) a	0.73 (0.06) a	0.07 (0.01) a
Bravo®	1.82 (0.06) c	0.17 (0.01) a	0.68 (0.06) a	0.08 (0.01) a
Sulfur w/ sticker	1.60 (0.06) ab	0.17 (0.01) a	0.70 (0.06) a	0.13 (0.01) b
Sulfur no sticker	1.78 (0.06) bc	0.19 (0.01) a	0.73 (0.06) a	0.11 (0.01) b
Sulfur ground	1.68 (0.06) abc	0.17 (0.01) a	0.70 (0.06) a	0.09 (0.01) a
P > F	0.0205	0.0942	0.9692	0.0015
	Ca %	**Mg %**	**Na %**	**B ppm**
Control	0.25 (0.05) a	0.09 (0.01) a	0.07 (0.05) a	14.03 (1.5) a
Bravo®	0.39 (0.05) a	0.12 (0.01) a	0.05 (0.05) a	17.55 (1.5) a
Sulfur w/ sticker	0.36 (0.05) a	0.10 (0.01) a	0.05 (0.05) a	17.03 (1.5) a
Sulfur no sticker	0.32 (0.05) a	0.09 (0.01) a	0.07 (0.05) a	18.08 (1.5) a
Sulfur ground	0.41 (0.05) a	0.12 (0.01) a	0.22 (0.05) a	14.98 (1.5) a
P > F	0.2144	0.2442	0.0841	0.3122
	Zn ppm	**Mn ppm**	**Fe ppm**	**Cu ppm**
Control	18.75 (3.3) a	317.5 (82.0) a	106.3 (22.5) a	5.25 (0.93) a
Bravo®	25.25 (3.3) a	391.8 (82.0) a	104.0 (22.5) a	6.50 (0.93) a
Sulfur w/ sticker	23.50 (3.3) a	342.5 (82.0) a	136.3 (22.5) a	6.50 (0.93) a
Sulfur no sticker	21.00 (3.3) a	462.5 (82.0) a	78.5 (22.5) a	7.25 (0.93) a
Sulfur ground	28.75 (3.3) a	523.8 (82.0) a	92.5 (22.5) a	6.75 (0.93) a
P > F	0.2941	0.4000	0.4870	0.6523

Table 3.5. 2000 foliar nutrients. Treatments associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatments	N %	P %	K %	S %
Control	1.55 (0.05) a	0.16 (0.01)a	0.71 (0.03)a	0.10 (0.003)a
Bravo®	1.60 (0.05)ab	0.17 (0.01)a	0.74 (0.03)a	0.11 (0.003)a
Sulfur w/ sticker	1.67 (0.05)abc	0.16 (0.01)a	0.70 (0.03)a	0.11 (0.003)a
Sulfur no sticker	1.77 (0.05)c	0.18 (0.01)a	0.72 (0.03)a	0.11 (0.003)a
Sulfur ground	1.70 (0.05)bc	0.17 (0.01)a	0.75 (0.03)a	0.11 (0.003)a
P > F	0.0435	0.1140	0.7057	0.0625
	Ca %	**Mn ppm**	**Mg %**	**Na %**
Control	0.39 (0.02)a	563.5 (83.9)a	0.13 (0.01)a	0.05 (0.01)a
Bravo®	0.40 (0.02)a	596.2 (83.9)a	0.13 (0.01)a	0.05 (0.01)a
Sulfur w/ sticker	0.41 (0.02)a	582.1 (83.9)a	0.13 (0.01)a	0.06 (0.01)a
Sulfur no sticker	0.39 (0.02)a	496.5 (83.9)a	0.11 (0.01)a	0.05 (0.01)a
Sulfur ground	0.39 (0.02)a	552.1 (83.9)a	0.13 (0.01)a	0.07 (0.01)a
P > F	0.9419	0.9321	0.5694	0.4157
	B ppm	**Zn ppm**	**Fe ppm**	**Cu ppm**
Control	17.8 (1.08)b	31.1 (4.98)a	66.5 (3.04)a	9.8 (0.50)a
Bravo®	23.3 (1.08)a	27.4 (4.98)a	64.3 (3.04)a	9.6 (0.50)a
Sulfur w/ sticker	18.1 (1.08)b	28.9 (4.98)a	66.8 (3.04)a	9.9 (0.50)a
Sulfur no sticker	19.2 (1.08)b	40.3 (4.98)a	67.7 (3.04)a	10.1 (0.50)a
Sulfur ground	18.0 (1.08)b	32.4 (4.98)a	65.2 (3.04)a	9.9 (0.50)a
P > F	0.0032	0.4103	0.9388	0.9709

3.3.5 PCR analyses

Bravo® was the most effective treatment in reducing the colonization of foliage by *PG* nine months after treatment (Figure 3.4).

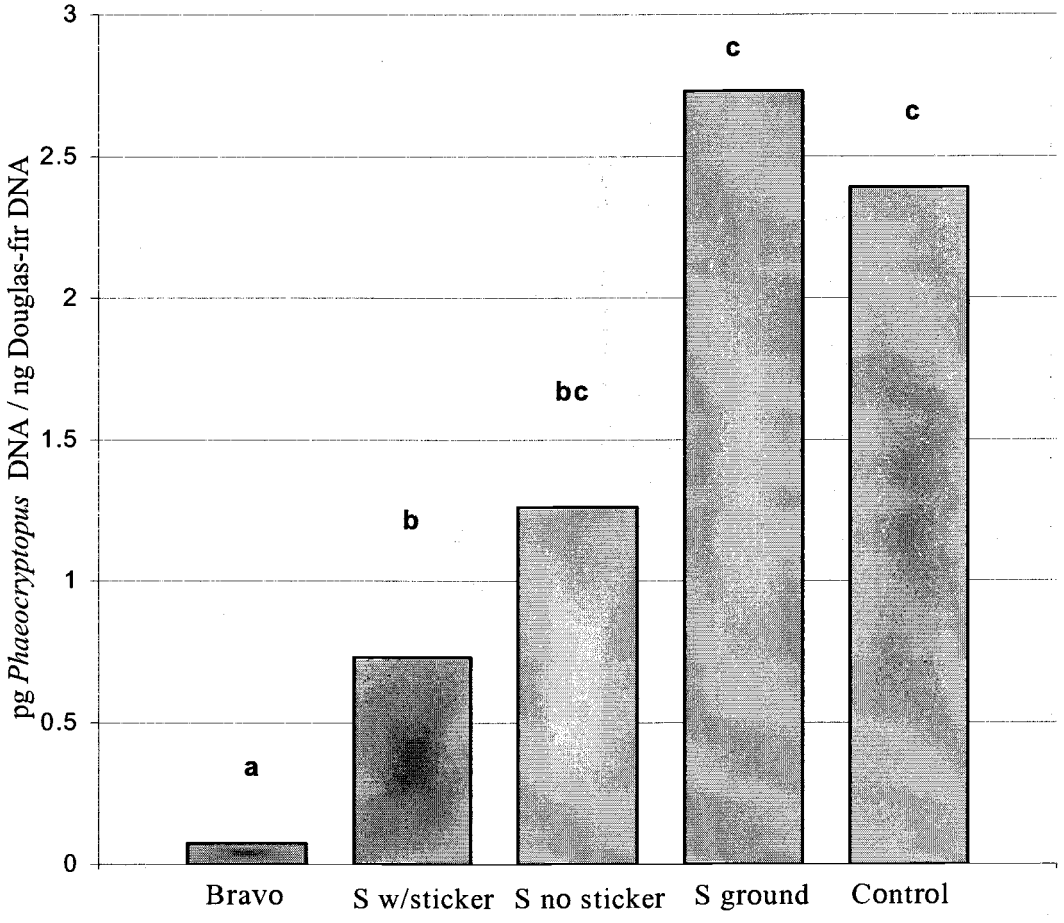


Figure 3.4. 1999 Polymerase Chain Reaction (PCR) results (*PG Phaeocryptopus* DNA / ng Douglas-fir DNA), 1999 needle cohort, sampled April 25, 2000. Foliage was sprayed in June-July 1999. Bars associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level.

The Bravo® treatment had a significantly decreased *PG* infection level when compared to all other treatments (p-value=.0001). The sulfur with-sticker was also significantly effective at lowering *PG* infection levels when compared to the control treatment (p-value=.005), but less effective than the Bravo® treatment. All other treatments were not significant in lowering *PG* infection levels when compared to the control treatment. However, the sulfur with-no-sticker treatment carried only half the infection level of the control treatment.

Based on samples taken in February 2001 (19 months after treatment), the 1999 cohort needles treated with Bravo® had significantly lower infection levels than the control treatment (p-value=.0001) (Figure 3.5). The sulfur with-no-sticker foliar application treatment had significantly lower infection than the sulfur ground treatment (p-value=.0330), which had the highest level of infection of all treatments. However, the sulfur ground treatment did not differ significantly from the sulfur with-sticker and control treatments (p-values=.4743 and .1954, respectively). The 2000 needle cohort showed no significant differences between treatments (p-value>.05) (Figure 3.6). However, the Bravo® treatment showed a trend of having the least amount of infection when compared to all other treatments.

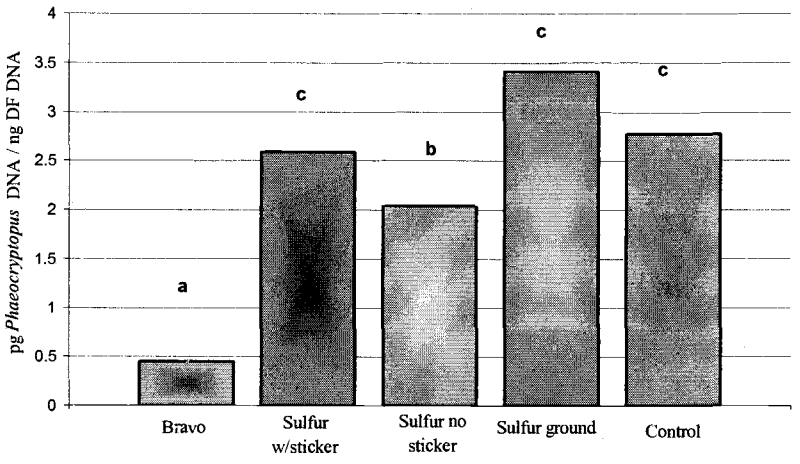


Figure 3.5. 2000 Polymerase Chain Reaction (PCR) results (pg *Phaeocryptopus* DNA / ng Douglas-fir DNA), 1999 needle cohort, sampled February 2001. Foliage was sprayed in June-July 1999. Bars associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level.

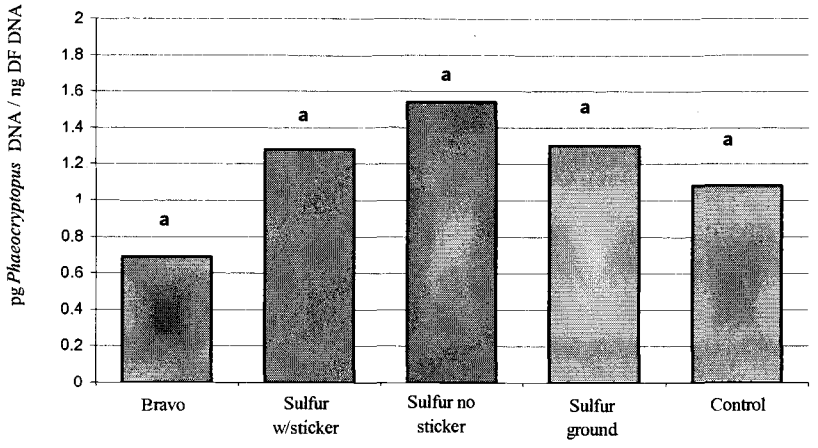


Figure 3.6. 2000 Polymerase Chain Reaction (PCR) results (pg *Phaeocryptopus* DNA / ng Douglas-fir DNA), 2000 needle cohort, sampled February 2001. Foliage was sprayed in June-July 1999. Bars associated with the same letter are not significantly different the $\alpha \leq 0.05$ level.

3.3.6 Sapling needle weights

There were no treatment effects on mean weight per 100 oven-dried needles for the 1999 cohort needles sampled during December of 1999 or the 2000 cohort needles sampled in January 2001 (Table 3.6). Dried needle weights varied considerably, which made detecting treatment differences difficult.

Table 3.6. 1999 and 2000 sapling needle weights (grams). Means within the same column associated with the same letter are not significantly different at the $\alpha \leq 0.05$ level. Standard error of the mean is in parentheses.

Treatment	1999 Cohort	2000 Cohort
Control	0.57 (0.05) a	0.58 (0.03) a
Bravo®	0.55 (0.05) a	0.57 (0.03) a
Sulfur w/sticker	0.49 (0.05) a	0.65 (0.03) a
Sulfur no sticker	0.63 (0.05) a	0.60 (0.03) a
Sulfur ground	0.58 (0.05) a	0.58 (0.03) a
P>F	0.3553	0.4364

3.3.7 Needle retention

The May 2000 needle assessment showed no significant differences among treatments with regard to the treated 1999 main needle cohort (p-value=.4337) (Table 3.7). Additionally, there were no significant differences among the main 1998, main 1997, lateral 1999, lateral 1998, and lateral 1997 branches. A partial

explanation for these results is that the majority of needles are not cast until the summer months during the second growing season.

The May 2001 needle assessment showed no significant difference among treatments for any main branches or laterals (Table 3.8). However, the treated main 1999 needle cohort showed an approximate increase of 19% needle retention for the Bravo® treatment when compared to the control treatment.

Table 3.7. Mean needle retention by treatment, sampled in May 2000. Treatments (1999) associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$. Standard error of the mean is in parentheses.

Treatment	Main 99	Main 98	Main 97	Lateral 99	Lateral 98	Lateral 97
Control	93.0 (2.2) a	8.5 (3.5) a	2.5 (1.3) a	95.0 (4.2) a	67.0 (10.6) a	15.0 (8.7) a
Bravo®	94.0 (2.2) a	9.0 (3.5) a	2.0 (1.3) a	94.5 (4.2) a	33.0 (10.6) a	9.5 (8.7) a
Sulfur w/ sticker	94.0 (2.2) a	11.5 (3.5) a	2.5 (1.3) a	95.0 (4.2) a	43.0 (10.6) a	16.7 (8.7) a
Sulfur no sticker	90.0 (2.2) a	12.5 (3.5) a	4.5 (1.3) a	89.0 (4.2) a	68.0 (10.6) a	27.8 (8.7) a
Sulfur ground	95.0 (2.2) a	12.0 (3.5) a	3.2 (1.3) a	94.5 (4.2) a	55.0 (10.6) a	26.9 (8.7) a
P > F	0.4337	0.8934	0.7182	0.8261	0.1036	0.5604

Table 3.8. Mean needle retention by treatment, sampled in May 2001. Treatments (1999) associated with the same letter within a column are not significantly different at the $\alpha \leq 0.05$. Standard error of the mean is in parentheses.

Treatment	Main 00	Main 99	Main 98	Lateral 00	Lateral 99	Lateral 98
Control	97.5 (2.4) a	69.0 (8.4) a	0.5 (5.3) a	99.5 (0.57) a	99.5 (7.0) a	32.0 (10.2) a
Bravo®	95.5 (2.4) a	84.5 (8.4) a	10.0 (5.3) a	100 (0.57) a	81.5 (7.0) a	12.5 (10.2) a
Sulfur w/ sticker	92.0 (2.4) a	69.5 (8.4) a	1.5 (5.3) a	99.5 (0.57) a	88.0 (7.0) a	27.0 (10.2) a
Sulfur no sticker	96.0 (2.4) a	78.5 (8.4) a	2.5 (5.3) a	98.5 (0.57) a	96.0 (7.0) a	33.0 (10.2) a
Sulfur ground	99.5 (2.4) a	70.5 (8.4) a	7.0 (5.3) a	100 (0.57) a	73.5 (7.0) a	32.8 (10.2) a
P > F	0.2662	0.6223	0.6748	0.3481	0.0723	0.5767

3.3.8 Vector diagrams

Vector diagrams constructed on the 1999 foliar samples showed an increase in relative concentration for all nutrients (N, P, Ca, S, Mg, Z, Cu, and B) except potassium over all treatments when compared to the control. Potassium concentration actually decreased over all treatments. Additionally, the relative needle dry weights increased only with the sulfur with-no-sticker application. Relative needle dry weights decreased slightly with the Bravo® treatment and more markedly with the foliar application of sulfur with-sticker.

Nitrogen and sulfur, the two nutrients that differed significantly among treatments, also showed large differences after vectoring. Sulfur showed an approximate 40% increase in content and a 30% increase in concentration for the sulfur with-no-sticker application when compared to the control (Figure 3.7).

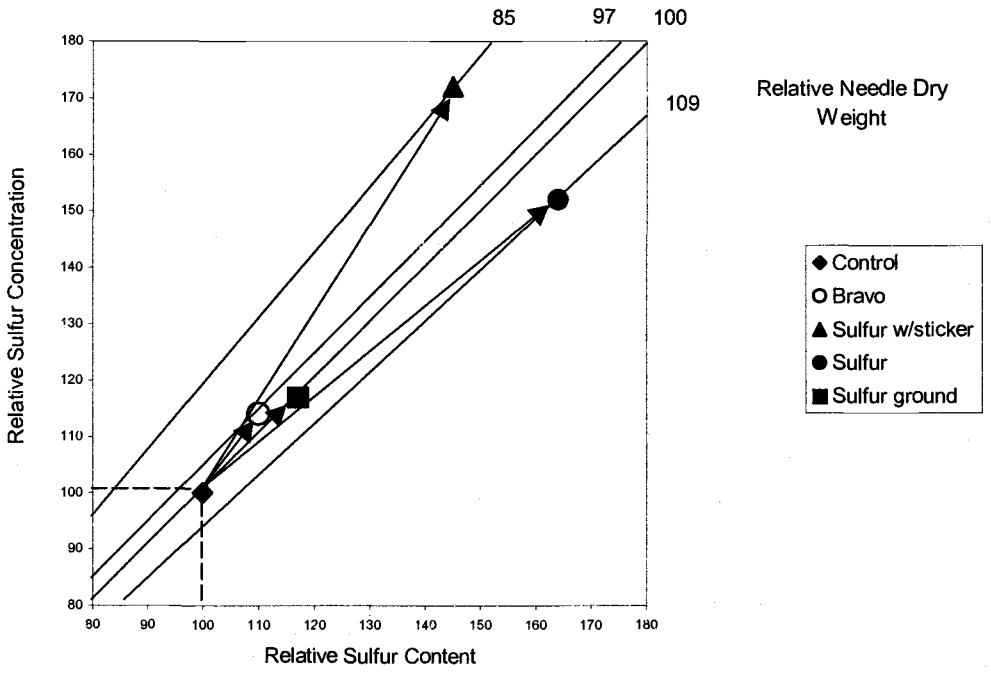


Figure 3.7. Relative sulfur shift six months after treatment. Sulfur levels among the sulfur with-sticker and sulfur with-no-sticker were significantly different from the control treatment ($p \leq 0.05$).

Additionally, the sulfur with-sticker treatment increased 30% in content and 40% in concentration. Both the Bravo® and sulfur ground treatments only increased slightly in both content and concentration.

Vectoring 1999 nitrogen levels showed that the Bravo® treatment increased relative content by 13% and relative concentration by 17% (Figure 3.8). The sulfur with-no-sticker application showed an increase in relative nitrogen content of 20%,

while the concentration was increased by 13%. The sulfur with-sticker and sulfur ground applications showed only marginal increases in relative nitrogen concentration. The sulfur with-sticker treatment actually showed a 10% decrease in relative nitrogen content while the sulfur ground application increased relative nitrogen concentration by 8%.

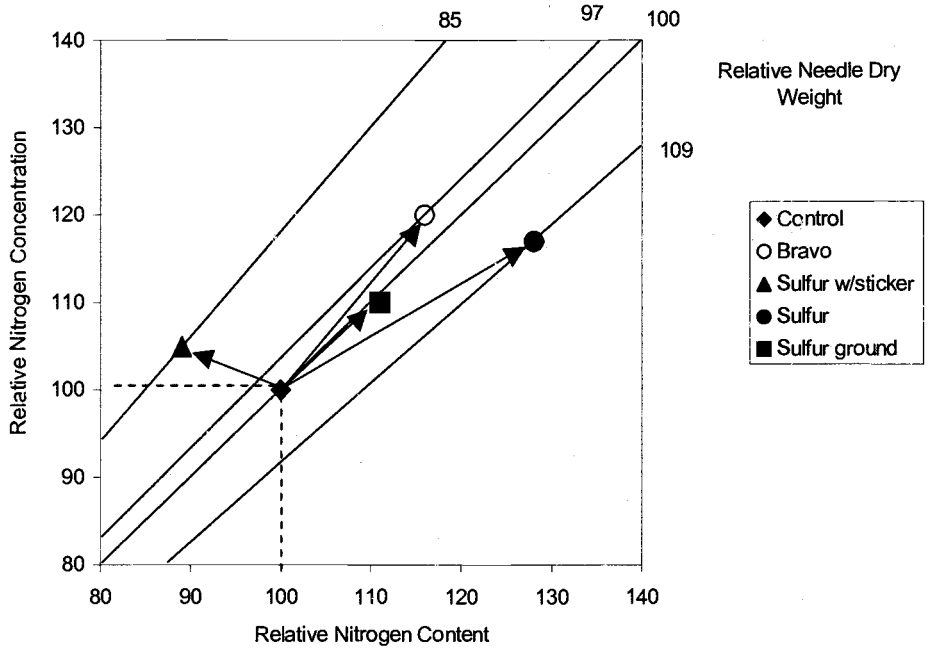


Figure 3.8. Relative nitrogen shift six months after treatment. Nitrogen levels between the Bravo® and sulfur with-no-sticker were significantly different when compared to the control ($p \leq 0.05$).

Vector analyses of the 2000 cohort foliar samples showed no shift upward with regard to relative needle dry weights when compared with the control treatment. Unit weights actually decreased with the Bravo®, sulfur with-sticker, and sulfur ground treatments. The sulfur with-no-sticker application remained consistent with the control treatment.

Nitrogen and boron were the only 2000 cohort foliar nutrients that were statistically different when compared to the control treatment (Section 4.4.4). This was also recognized after the construction of both respective vector diagrams. The relative nitrogen concentration increased for all treatments when compared with the control treatment (Figure 3.9).

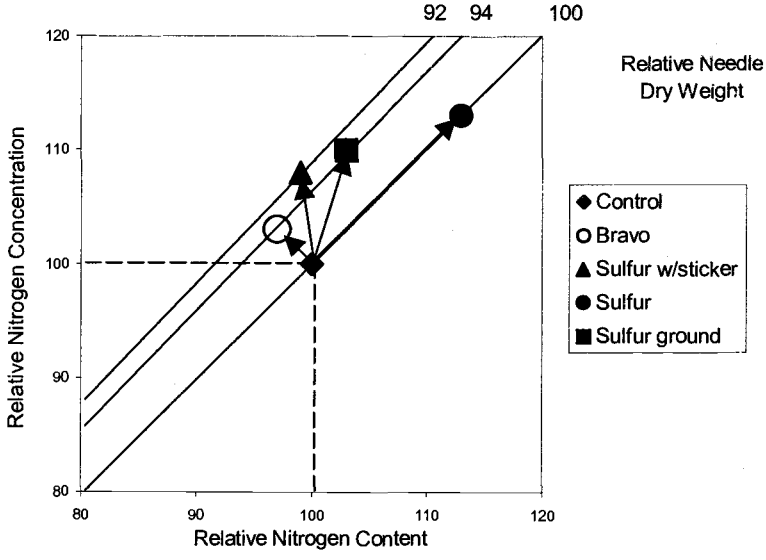


Figure 3.9. Relative nitrogen shift 19 months after treatment (2000 needle cohort). Nitrogen levels among the sulfur ground and sulfur with no sticker treatments were significantly different from the control treatment ($p \leq 0.05$).

The sulfur with-no-sticker showed the largest increase in nitrogen concentration (10%) as well as content (12%). The Bravo® treatment increased marginally in nitrogen concentration, but decreased in content. The sulfur with-sticker treatment and sulfur ground treatments remained relatively the same in nitrogen content but increased approximately 10% in concentration.

The boron vector diagram shows that the Bravo® treatment, and to a lesser extent the sulfur with-no-sticker treatment increased in both relative boron content and concentration (Figure 3.10).

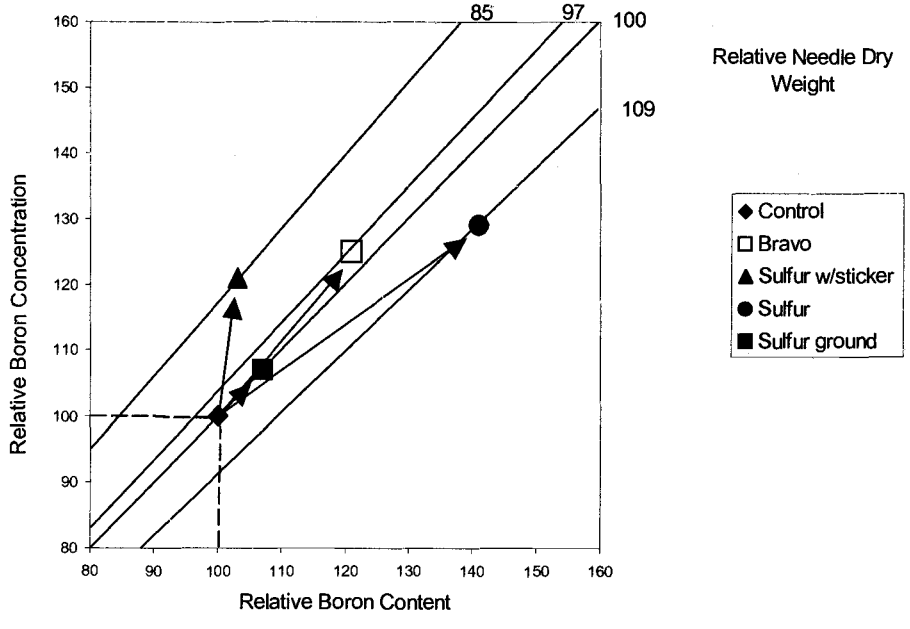


Figure 3.10. Relative boron shift six months after treatment. Boron levels among all treatments are not significantly different ($p \leq 0.05$).

The Bravo® treatment increased relative content by 17% and concentration by 23%. The other treatments remained consistent with the control.

After constructing the 2000 foliar sulfur vector diagram, a trend was recognized as an increase in relative sulfur concentration was observed in all treatments relative to the control. Additionally, the sulfur with-sticker application showed a relative content increase of 9%. The sulfur with-sticker increased the most in relative concentration at 11%. All other treatments remained constant with the control in regard to relative content. All other nutrients that had vector diagrams constructed showed no distinct patterns (phosphorus, potassium, calcium, magnesium, zinc, and copper) (data not shown).

3.4 Discussion

DBH and height growth did not differ significantly among respective treatments after the first or second growing season. Based on infection results this is not surprising. Perhaps if more than one-year worth of treatments was implemented, a difference in growth may have been recognized with a larger increase in photosynthetic area of the tree due to an increase in the retention of needle cohorts. It can be concluded that no treatment had an effect on DBH or height growth after one and/or two full growing seasons.

Based on field trials it appears that Bravo® is an effective treatment in reducing *PG* infection levels in Douglas-fir nine months after treatment at a rate of

3.75 pts/100 gal when sprayed on the foliage three different times at two week intervals during shoot elongation (the highest infection period). This is consistent with published results from studies on SNC in Douglas-fir Christmas trees, which have shown Bravo® to be effective in reducing infection by *PG* (Chastagner and Byther, 1982).

Sulfur with-sticker also significantly reduced *PG* infection. The amount of *PG* colonization in this treatment group was intermediate between the Bravo® and sulfur with-no-sticker. The amount of *PG* DNA detected was approximately ten times greater in the sulfur with-sticker treatment than for Bravo®. The sulfur with-no-sticker was intermediate between the sulfur with-sticker and the control treatment. Sulfur applied to the ground had no effect on reducing colonization by *PG*. Sulfur effectively reduced ascospore germination and hyphal growth of *PG* in culture studies, and therefore it is likely that reduced foliage colonization was due to a contact fungicidal effect (Stone *et al.*, 2000). This is most likely why the sulfur with-sticker was significant in reducing infection and sulfur with-no-sticker was not. Between treatment applications there was precipitation that could have inhibited the effectiveness of the sulfur with-no-sticker treatment. This precipitation would reduce the amount of active elemental sulfur on the needle surface, thus decreasing the efficacy of the treatment.

The 1999 foliar nutrient results demonstrate that after six months from treatment there is a significantly higher percentage of sulfur on the trees where sticker was used. However, because foliar samples were not washed before

nutrient analysis, it is not possible to say if any or all of the sulfur was absorbed into the needle. It may be that there was sulfur residue on the needle surface after this six month time period.

After 19 months the 1999 needle cohort that had been treated with Bravo® remained significantly different from the other respective treatments with respect to lower infection levels. However, the sulfur with-sticker and sulfur with-no-sticker treatments did not differ significantly from the control after this extended period of time. The reason for this is that despite having infection levels that were significantly reduced when compared to the control treatment, there was fungus that did colonize the needle. This fungus continued to grow throughout the following year, thus increasing its' presence within individual needles. Therefore, the significant difference in infection reduction that was seen after the initial nine-month period was not seen after 19 months. This demonstrates the need for further research dealing with rates of elemental sulfur that could potentially carryover significant infection reductions two years after treatment when looking at the same needle cohort.

There were no significant differences among needle retention two years after the treatments were applied. This is not overly surprising because the majority of needles would tend to be cast during the summer months two years after treatment, and the survey was conducted in May (before the next shoot elongation). However, a trend is developing in that the Bravo® treatment has increased needle retention approximately 19% on the main 1999 needle cohort

when compared with the control treatment. This gap would be expected to increase over time because the 1999 cohort that was treated with Bravo® is nearly free of *PG*, while the control is heavily infected, thus causing casting of this cohort during the summer of 2001. An assessment the following year would validate this assumption.

When looking at both the 2000 and 2001 needle retention figures (see figures 3.11 and 3.12) a dramatic difference is seen between the retention of the second year cohort needles over all treatments. The increased retention in the 2001 figure can be attributed to less moisture during the period of time where susceptibility to infection was the greatest (June and July 2000). Additionally, the mild winter of 2000-2001 resulted in less wind-damage and needle loss.

Vectoring substantiated the statistical results of significance for foliar nutrient samples during 1999 and 2000. In addition, it was possible to see trends in nutrients that were not of statistical significance. For example, sulfur in the 2000 cohort showed moderate increases in the sulfur with-sticker treatment for both relative content and concentration. This is of importance due to the relationship between nitrogen and sulfur and nutrient imbalances that increase free amino acids, and thus the food supply for *PG*. Being able to recognize and understand trends can lead to better research in the future.

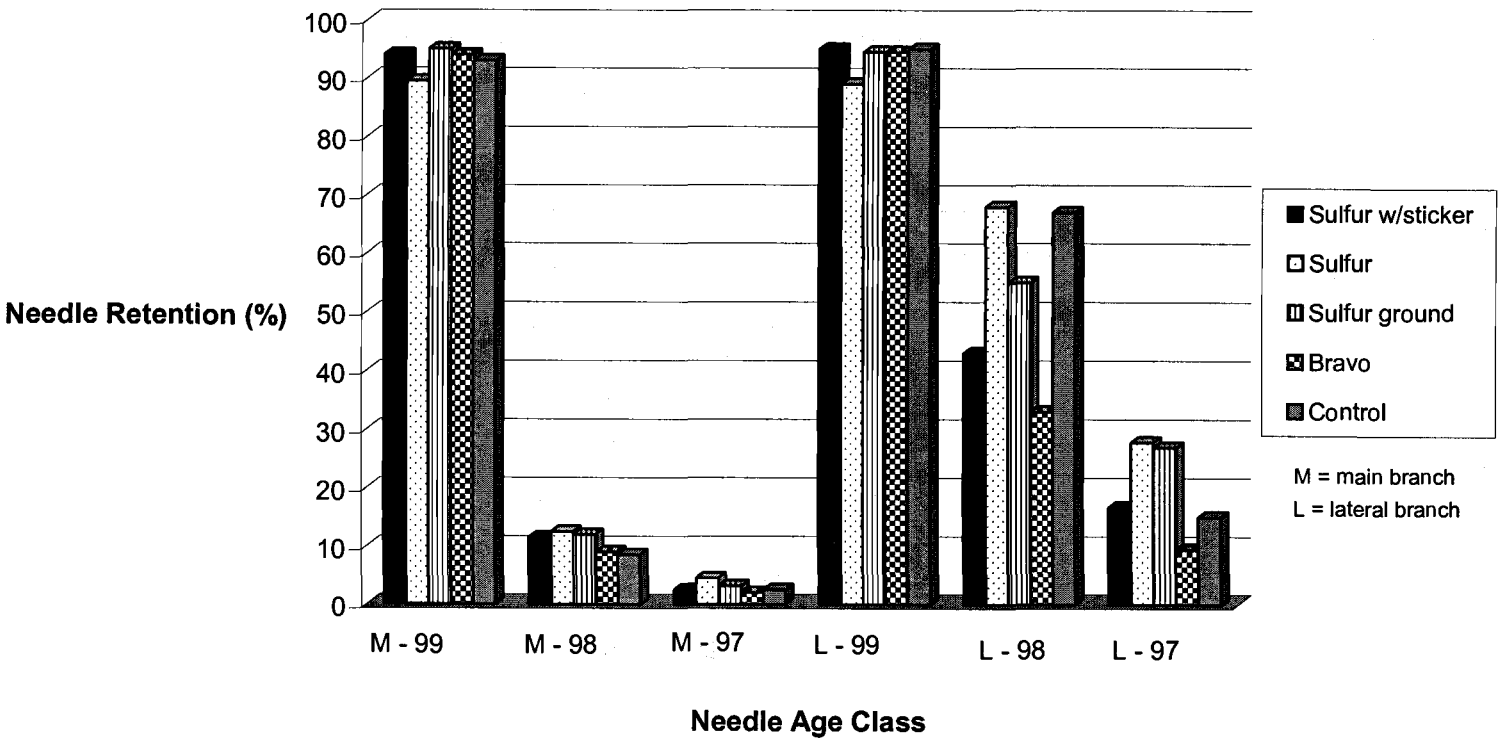


Figure 3.11. Needle retention assessment by treatment, May 2000.

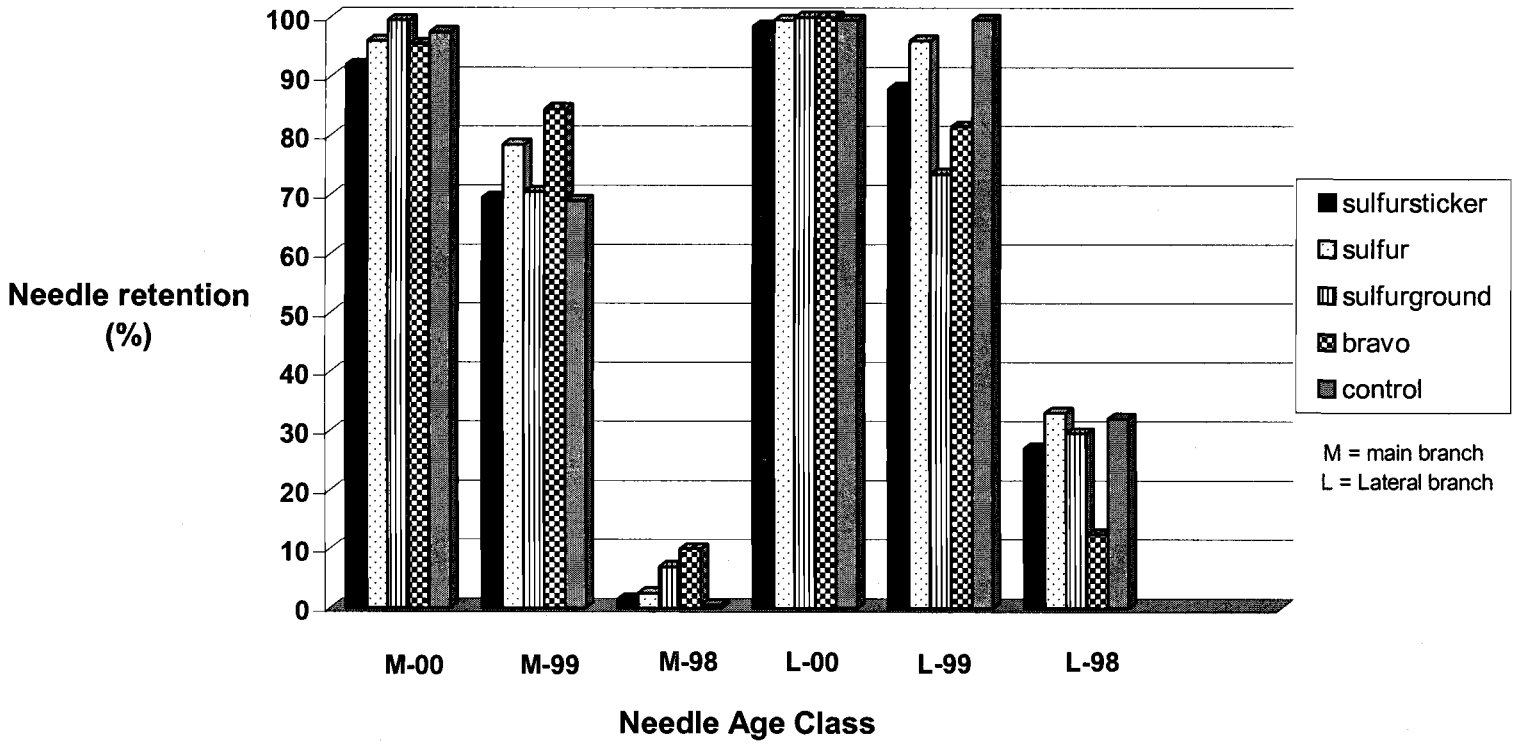


Figure 3.12. Needle retention assessment by treatment, May 2001.

3.5 Conclusions and recommendations

Based on the aforementioned results, the use of Bravo® as a means to decrease *PG* infection on an individual tree basis is effective. However, Bravo® is registered for use only in agricultural situations. Therefore, any forestry use of Bravo® is prohibited without an experimental use permit.

Trends showed that sulfur (Thiolux®) with-sticker applied as a foliar application was significant in decreasing *PG* in the first growing season. However, the fungus that was able to colonize the needle in the first year (even though it was at a significantly decreased level from the control treatment) continued to grow throughout the following year. This response negated any positive effects from the initial treatment, thus rendering Thiolux® at the rates and dosages used as an ineffective way to decrease *PG* infection over several years.

More research on rates and timing of applications are needed to determine if elemental sulfur can be used effectively to decrease *PG* infection on an individual tree and stand level basis. Opportunities exist to further study the potential nutrient effects of elemental sulfur over an extended period of time. Additionally, Thiolux® for forestry use is currently registered as a mineral but not a fungicide.

3.6 Acknowledgements

This research was funded through the Swiss Needle Cast Cooperative at Oregon State University – a consortium of industrial, federal, state, and tribal landowners in Oregon and Washington. Dr. Jeff Stone and Wendy Sutton were instrumental in carrying out PCR analyses. The Timber Company (now Plum Cr. Timber Co.) generously provided the land for this research. The use of trade names is for information and convenience of the reader and does not constitute official endorsement or approval by Oregon State University.

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Chapter 4

Chlorophyll Fluorescence as a Tool for *Phaeocryptopus gaeumannii* Quantification on an Individual Tree Basis

by

Gabriel A. Crane

4.0 Abstract

The objective of this study was to evaluate the usefulness of quantum yield (measured via chlorophyll fluorescence) as a tool to detect differing levels of *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*) infection, causal agent of Swiss needle cast (SNC), in the needles of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) saplings and seedlings. Quantum yield was measured on a Douglas-fir sapling sulfur study and on an open-pollinated potted Douglas-fir seedling study using a modulated chlorophyll fluorometer. Varying levels of *PG* infection in the sulfur study were created by applying foliar and ground applications of elemental sulfur and foliar applications of Bravo® (chlorothalonil) fungicide. A control treatment in which *PG* colonization was not controlled was also implemented. In the Douglas-fir potted seedling study different levels of *PG* infection were obtained by increasing or decreasing the inoculation time. *PG* infection levels were determined using the Polymerase Chain Reaction (PCR) procedure for the sulfur study saplings and by using pseudothecia density (%) on stomatal rows for the potted seedlings. For the Douglas-fir potted seedlings there was a significant relationship (R^2=.43) between pseudothecia density and quantum yield. As the percent pseudothecia increased, there was a decrease in the measured yield values. No significant differences in quantum yield were found among respective treatments (i.e. *PG* infection levels) in the sulfur study saplings. Additionally no relationship between PCR and quantum yield was established.

4.1 Introduction

A gap in Swiss needle cast (SNC) research is the lack of a consistent, objective, quantification of *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*) infection on an individual tree basis. Chlorophyll fluorescence may be an ideal test for this purpose because it is inversely related to the rate of photosynthesis, thus more severely infected and photosynthetically inactive plants yield higher fluorescence emissions. Chlorophyll fluorescence is defined as the electromagnetic radiation emitted when a chlorophyll molecule goes from an excited singlet state reached by absorption of red light (680nm) to a singlet ground state (Nobel, 1991). Krause and Weis (1984) promote the potential of chlorophyll fluorescence as a good measure of a plant's physiological status. Additionally, Lambers *et al.* (1998) promote chlorophyll fluorescence as a useful technique to quantify effects of stress on photosynthetic performance.

Under optimum conditions of photosynthesis, the dissipation of absorbed light energy via chlorophyll fluorescence is minimal. However, when plant conditions change, chlorophyll fluorescence emissions can also change. It can be assumed that factors that affect the photosynthetic rate (as would be expected from *PG* infection) will produce corresponding changes in fluorescence emissions. Thus, photosynthetic activity of the plant and its responses to disturbances can be measured effectively.

Chlorophyll fluorescence is a bi-product of photosynthetically active pigment molecules. For photosynthesis to occur, there must be absorption of light energy by chlorophyll, as well as other photosynthetically active pigments in the chloroplasts (Larcher, 1995). Antennal pigments absorb light quanta of which only a small fraction of the light energy is used in a complex set of reactions to generate NADPH2 and ATP from NADP+ and ADP. The remaining light energy is emitted as heat, and fluorescent and phosphorescent light (Larcher, 1995). It has been observed that 3-9% of the light energy absorbed by light harvesting pigments is remitted as fluorescence (Opti-Sciences, 1997).

The quantity, Fvar/Fmax has been widely used as a measure of photochemical efficiency or optimum quantum yield of photosystem II (Mohammed *et al.*, 1995). Quantum yield is a percentage that is calculated from the amount of energy emitted from an excited molecule to the total way the molecule loses the energy (Christian, 1986). Fvar/Fmax is the ratio of the "variable" to maximal fluorescence. It appears that Fvar/Fmax correlates well with the quantum yield of photosynthesis measured as O_2 production or CO_2 uptake at low irradiance (Lambers *et al.*, 1998). A decrease in Fvar/Fmax can be due to a decrease in Fmax and/or an increase in Fo (minimal fluorescence).

Chlorophyll fluorescence has been a useful tool in ecophysiological studies for detecting changes in the photosynthetic apparatus in connection with stress (Larcher, 1995). Larcher (1995) define stress as a significant deviation from the conditions optimal for life, and eliciting changes and responses at all functional

levels of the organism which, although at first reversible, may also become permanent. Additionally, chlorophyll fluorescence is a non-destructive and non-invasive measure of a plants physiological viability (Bolhar-Nordenkampf *et al.*, 1989). Therefore, if *PG* infection can be detected through fluorescence measurements and the degree of infection determined, then fluorometry could become a useful tool in determining *PG* infection on an individual tree and stand level basis.

4.2 Objectives

The objective of this preliminary study was to determine if quantum yield measurements (measured via chlorophyll fluorescence) were correlated with *PG* infection levels on an individual tree basis. The null hypothesis of this study is that the overall photochemical quantum yield is not significantly correlated with *PG* infection level (determined by the Polymerase Chain Reaction (PCR) procedure and pseudothecia density).

4.3 Materials and methods

Quantum yield of photosynthetic efficiency measurements were made at two previously installed studies, namely the sulfur sapling and Douglas-fir

(*Pseudotsuga menziesii*) (Mirb.) Franco) potted seedling studies where individual sample trees had varying levels of *PG* infection.

4.3.1 Sulfur study

The sulfur study site was installed near Toledo, Oregon in an existing six-year old Douglas-fir plantation in May 1999. The plantation was moderately to heavily affected by SNC (needle retention <2 years). The plantation had a stocking density of 310 trees/acre. The soils are Tolovana of the Reedsport Association. The site index (50) is 136. The study was implemented on industrial timber land owned by The Timber Company (currently Plum Creek Timber Co.).

This experiment utilizes a complete randomized design with 10 replications (trees) for each of the five treatments. Treatments were assigned randomly to trees utilizing the entire site. Selected trees were at least 6 m apart to avoid treatment drift from nearby applications. Trees with forked stems or which originated from natural regeneration were not used for measurement trees. Each tree was clearly identified with an aluminum tag, marking paint, and flagging.

There are five treatments in the study:

1) Untreated control
2) Bravo®(chlorothalonil) fungicide @ 3.75 pts/100 gal sprayed on the foliage
3) Sulfur (Thiolux®) diluted with water (25 lb per 100 gal) sprayed on the foliage

4) Sulfur (Thiolux®) diluted with water (25 lb per 100 gal) with TacTic® sticker (8 oz per 100 gal) sprayed on the foliage
5) Sulfur (Thiolux®) diluted with water (25 lb per 100 gal) sprayed on the ground under each tree within the drip line

Treatments were applied on June 8, June 25, and July 10, 1999 using a truck tank sprayer at 38 psi. Each tree was sprayed evenly top to bottom for 14 seconds resulting in an application rate of 2 oz Thiolux® per tree. This resulted in a "wetting" of the needle surface area. Treatments were applied at two-week intervals that corresponded with shoot elongation. This is because the peak period for needle infection occurs during the time of elongation when there is new needle growth and plentiful moisture creating ideal infection conditions. It is important to note that a needle can only become infected during the first three months following elongation. After this, the needle is resistant to fungal colonization (Personal communication, Dr. Jeff Stone, Oregon State University, 2000). Thiolux® is a dry flowable micronized sulfur. The micronized or small particle formulation is designed to provide more thorough spray coverage. The Thiolux® treatments are referred to as sulfur treatments throughout this paper.

4.3.2 Douglas-fir potted seedling study

The Douglas-fir potted seedlings were a subset from another SNC study. The Douglas-fir seedlings (Starker Forests Inc., Corvallis, OR, USA) were open-pollinated and had differing levels of *PG* infection. Initially, the 1-0 seedlings were

obtained from a container nursery and had not previously been exposed to *PG* inoculum. Various levels of *PG* inoculation were achieved by placing seedlings under the canopy of heavily *PG*-infected Douglas-fir trees. To vary rates of infection, seedlings were exposed to *PG* inoculum for either two-week, four-week, or eight-week intervals near Tillamook, Oregon (Salal plot, see Stone *et al.*, 2000). Peak spore release at this site occurred in June 1999. Seedlings exposed in May had no *PG* infection, and those incubated in June had increasing infection with increasing incubation times (J.K. Stone, unpublished). PCR analyses verified that there were different levels of infection based on exposure time to inoculum. At the end of the exposure periods, the trees were returned to the OSU Botany farm for incubation. In addition, a control group was initially kept at the OSU Botany farm to remain disease free. At the time of fluorescence measurements the seedlings were three-years old.

4.3.3 Measurements and sampling

At the sulfur study site, quantum yield was measured on 50 Douglas-fir saplings with varying levels of *PG* infection. On each individual tree, three separate needles were sampled at mid-canopy using the current year needles. *PG*-infection levels were pre-determined on an individual tree basis using the PCR procedure (Winton *et al.*, 2001).

Measurements of the potted seedling study consisted of measuring 24 individual seedlings. Quantum yield was measured on three current-year needles

for each seedling. The total sample size was 72. The current year needle cohort was used on every seedling.

PG infection levels for the Douglas-fir potted seedling study group were determined by measuring pseudothecia density (%) on each individual measure needle. Initially, measured needles were placed on note cards with double-sided sticky tape and frozen in order to facilitate microscopic examination at a later date. The use of a microscope allows for a more accurate assessment of disease severity based on the number of pseudothecia present on stomatal rows.

Estimates of the percentage of needle stomata that were occluded with pseudothecia for each branch were calculated by averaging pseudothecia counts from three positions on each needle (one on each longitudinal third of the needle) (Manter *et al.*, 2000). Pseudothecial counts were conducted by visually counting the number of pseudothecia emerging from 100 consecutive stomata from the first complete row closest to the needle midrib (Manter *et al.*, 2000). All quantum yield measurements calculated via chlorophyll fluorescence (Y=(Fms-Fs)/Fms) were measured with the OS5-FL Modulated Fluorometer (Opti-Sciences, 1997). It is a versatile measuring instrument designed to measure chlorophyll fluorescence under a wide array of environmental conditions.

4.3.4 Statistical analysis

Data were analyzed for the sulfur study measurements using analysis of variance (ANOVA) to determine if the quantum yield measurements differed

among the respective treatments (varying levels of *PG* infection). Regression analyses were performed to determine if there was a relationship between quantum yield measurements and PCR levels at the sulfur study site. For the potted seedling study, regression analyses were performed to determine if there were relationships between quantum yield measurements and pseudothecia density (%). All data was analyzed using Statistical Analysis Software® (SAS Institute Inc., 1999).

4.4 Results

4.4.1 Sulfur study

Quantum yield measurements were not significantly different among the treatments (p-value=.3444) (Figure 4.1).

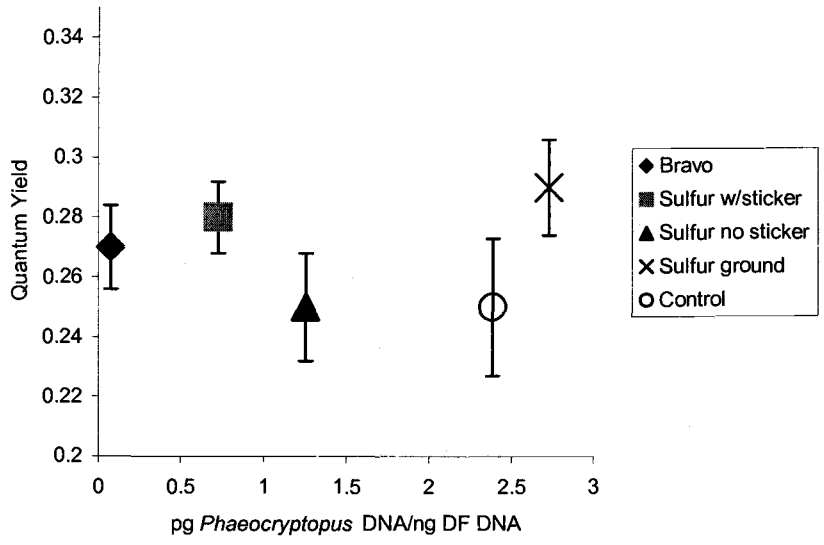

Figure 4.1. 2000 quantum yield and related PCR measurements, 1999 needle cohort (sulfur study site). Error bars are the standard error of the mean.

Additionally, there was no significant correlation between *PG* infection level and quantum yield measurements (p-value=.5822, R^2=0.01) (Figure 4.2).

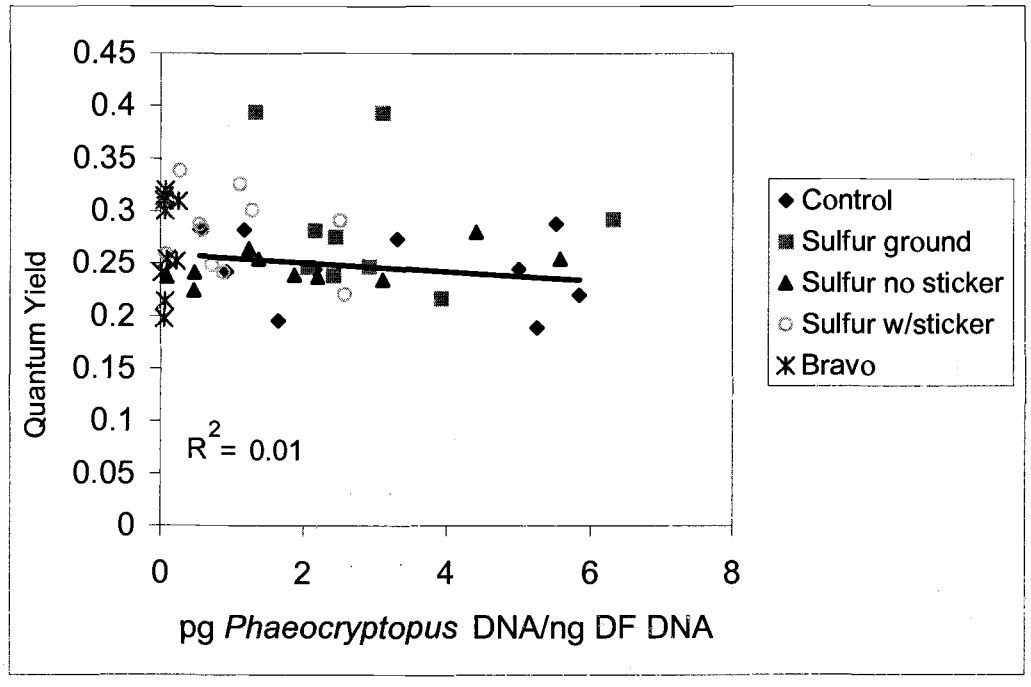

Figure 4.2. Relationship between pg *Phaeocryptopus* DNA/ng DF DNA (1999 needle cohort) and quantum yield measured via chlorophyll fluorescence at the sulfur study site.

4.4.2 Douglas-fir potted seedling study

For the Douglas-fir potted seedling study there was a significant negative correlation between pseudothecia density and quantum yield (R^2=.43) (Figure 4.3).

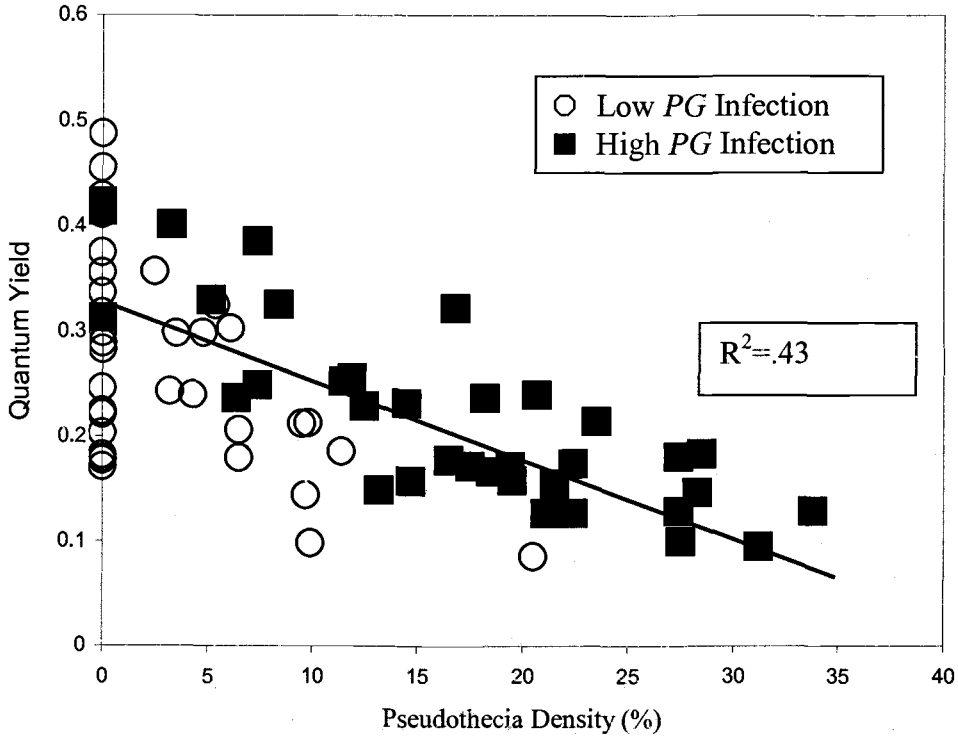

Figure 4.3. Relationship between pseudothecia density (%) (1999 needle cohort) and quantum yield measured via chlorophyll fluorescence at the Douglas-fir potted seedling study.

4.5 Discussion

At the sulfur study field site there were no significant differences in quantum yield among any of the five respective treatments. This was somewhat surprising due to the large variation among individual trees detected by the PCR analyses. However, PCR measurements can be variable and are known to differ somewhat on individual trees depending on where and how many needles are

sampled (personal observation). For example, in this particular study the control treatment had a mean infection level of 3.14 pg *Phaeocryptopus* DNA/ng DF DNA with a standard deviation of 2.1. Where infection was controlled with the Bravo® treatment the mean value was reduced to .10 with a standard deviation among ten samples being .08. Not only is there within tree variation, but also variation from tree to tree caused by multiple factors including, but not limited to microclimate, aspect, and genetic resistance. Additionally, because the same needles that were used for quantum yield measurements were not the same needles used for PCR analyses, more variation may have been introduced. The coefficient of variation in this study was 76.4. Despite the best efforts to sample needles with the same amount of light exposure on each tree, self-shading could potentially have been a source of variation as well. Thus, the relationship between the amount of *PG* and quantum yield measurements were not correlated in this particular study.

It would be highly recommended in future studies to measure quantum yield and PCR on the same set of needles in order to decrease variation. Additionally, taking several sub-samples per tree might increase overall precision and accuracy. It would have been beneficial to record pseudothecia density (%) on the sampled needles as well, despite the fact that past research has shown pseudothecia density and quantitative PCR as measures of fungal colonization to be significantly correlated with each other and with disease symptoms (Manter, 2001).

Quantum yield measurements taken from the potted seedling study revealed a significant relationship between pseudothecia density (%) and quantum yield

(R^2=.43). This suggests that when infection levels are determined using pseudothecia counts, a relationship can be established between infection levels and quantum yield. This makes sense due to the fact that *PG* colonization and subsequent infection causes an indirect increase in stomatal resistance due to pseudothecia formation and growth in individual stoma.

A reduction in photosynthesis occurs because the rate of CO_2 diffusion decreases into the leaf as well as internal CO_2 concentrations, which limits both the kinetics and activation of rubisco (Manter, 2001). CO_2 is the source of all carbon to be used for the growth of terrestrial plants and rubisco is the enzyme that mediates the incorporation of CO_2 into the photosynthetic process (Manter, 2001). Manter *et al.*, (2000) found that following pseudothecia emergence, stomatal conductance and carbon assimilation are both reduced in infected needles. However, Manter *et al.* (2000) make the inference that *PG* infection appears to have no direct impact on the level and function of energy capture within chloroplasts associated with changes in chlorophyll fluorescence. This is in contrast to the findings of the Douglas-fir potted seedling data, which suggest that quantum yield is reduced as infection levels (pseudothecia) increase.

4.6 Conclusion

The null hypothesis that quantum yield (measured via chlorophyll fluoresescence) does not correlate with *PG* levels measured by PCR was rejected. The hypothesis that quantum yield does not correlate with pseudothecia density was not rejected.

Determining the health and vigor of Douglas-fir trees with varying levels of *PG* infection (based on pseudothecia counts) through chlorophyll fluorescence measurements may provide an additional way to gain a better understanding of how individual trees respond to the stresses of SNC. However, to gain consistent results that will make this a viable field measurement tool, further research into the relationship between quantum yield and varying levels of *PG* infection is needed. In addition, it is necessary to refine the significances of these studies by exploring a measurement methodology that will reduce within tree infection variation and self-shading of needles.

4.7 Acknowledgements

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Chapter 5

Thesis Summary

5.0 Thesis summary

This research was conducted to understand the effects of fertilization, vegetation control, and elemental sulfur on Swiss needle cast (SNC) and the subsequent growth in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) saplings. Additionally, quantum yield (measured via chlorophyll fluorescence) was evaluated as a means to determine *Phaeocryptopus gaeumannii* (Rhode) Petrak (*PG*) infection level differences on an individual tree basis.

Chapter 1 is an introduction to SNC and *PG*. The current history and spread of the disease is outlined. Following the introduction is a general literature review describing *PG* and the current state of knowledge about its' infection biology.

Chapter 2 describes the effects of fertilization and vegetation control on *PG* infection and growth of coastal Douglas-fir saplings. Fertilization treatments had no effect on height or DBH growth after one full growing season. Additionally, fertilization alone had no significant effects on infection levels or foliar nutrients. Vegetation control on the other hand was responsible for a 16% to 19% increase in DBH growth depending on site. However, no differences in height growth were

recognized. Vegetation treatments alone had no significant impacts on *PG* infection levels. The site nearest the Willamette Valley fringe (Charlie Olson) exhibited an 8.4% increase in foliar nitrogen concentration due to vegetation control. The other two study sites showed a similar trend, in that the Bushy Peterson site had a 4.3% increase and the South Drake site a 4.5% increase in foliar nitrogen concentration. At all sites there were no consistent foliar nutrient concentration responses to fertilization other than for boron.

In an effort to understand how elemental sulfur (Thiolux®), and Bravo® (chlorothalonil) affect *PG* infection levels and the subsequent growth in Douglas-fir saplings a field study was implemented near Toledo, Oregon. Additionally, foliar and soil nutrient change and needle retention were of interest. A significant decrease in *PG* infection was recognized for the Bravo® applied at a rate of 3.75 pts/100 gal of water and the sulfur with-sticker applied at 25 lbs/100 gal of water (8 oz sticker/ 100 gal). However, the Bravo® treatment was 10 times more effective at lowering *PG* infection than was the sulfur with-sticker treatment. This is consistent with other SNC research, which has shown Bravo® to be highly effective in reducing *PG* colonization (Chastagner and Byther, 1982). In culture studies, Thiolux® has effectively reduced ascospore germination and hyphal growth (Stone *et al.*, 2000). This suggests that the reduced *PG* colonization on the treated needle cohort is due to a contact fungicidal effect.

The 1999 needle cohort foliar nutrient results show a significantly higher percentage of sulfur on needles where sticker was added to the sulfur treatment.

This is most likely why the sulfur with-sticker treatment was more effective in limiting *PG* colonization than was the sulfur treatment with-no-sticker. However, 19 months after treatment the 1999 needle cohort that had been treated with Bravo® was the only treatment to remain significantly different for the control treatments. Therefore, despite the sulfur with-sticker lowering initial infection it was not enough to keep the fungus from growing within the needle over an extended period of time. This demonstrates the need for further research dealing with rates of elemental sulfur that could potentially carryover significant infection reductions two years after treatment.

The Bravo® treatment increased foliar nitrogen levels by 20% nine months after the initial treatment when compared to the control treatment. However, the 2000 foliar nitrogen levels did not differ among treatments. The foliar treatment applications of both sulfur with (+86%) and without sticker (+57%) led to significantly increased levels of foliar sulfur.

Needle retention among treatments was not significantly different on the treated needle main or lateral branches 19 months after the initial treatment. Height and DBH were not significantly affected by any treatment after two growing seasons.

In Chapter 4 the usefulness of quantum yield (measured via chlorophyll fluorescence) was evaluated as a tool to detect differences in *PG* infection on an individual tree basis. Based on quantum yield measurements from two different study areas that had varying levels of *PG* infection, results were variable. At the

sulfur study site, no significant differences among treatments were found with respect to quantum yield measurements. Additionally no relationship between PCR and quantum yield was recognized. In contrast to the sulfur study, the Douglas-fir potted seedling study data showed a relationship between infection level (pseudothecia density %) and quantum yield.

In conclusion, it was found that vegetation control had a positive effect on DBH growth, as well as causing an increase in foliar nitrogen. No difference in *PG* infection was recognized. Fertilizer treatments were not effective in altering the majority of all parameters measured as discussed previously in Chapter 2. Foliar applications of sulfur (Thiolux®) with sticker at a rate of 25 lbs/100 gal of water (8 oz sticker/100 gal) reduced the initial colonization of *PG* colonization after one year.

No growth differences were recognized from any treatment. Quantum yield when used in conjunction with PCR as a relative scale of infection was shown not to be a viable method of *PG* infection level detection. However, when measured using pseudothecia density (%) as the scale of infection, a significant relationship was developed.

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