AN ABSTRACT OF THE THESIS OF

Todd M. Birchler for the degree of Master of Science in Forest Science presented on September 18, 1997. Title: Fall Fertilization Effects on Douglas-fir Seedling Quality.

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Abstract approved: _____

Robert W. Rose, Jr.

Coastal Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco) 1+1 seedlings were fertilized with two types of fertilizers (NH_4NO_3+K_2SO_4 and $(NH_4)_2SO_4$+KCl) at four rates (0, 80, 160, 320 kg N and K/ha) split over 3 application dates (September 19, October 13, November 1, 1996). By January 10, total Kjeldahl nitrogen (TKN) concentrations increased 16, 30, and 34% and contents increased 6, 20, and 26% for the 80, 160, and 320 kg N/ha treatments relative to the unfertilized seedlings. Potassium levels remained relatively unchanged as a result of the fertilization treatments. Chloride concentrations increased 57, 77, and 112% and contents increased 45, 71, and 92% for the 80, 160, 320 kg K/ha as KCl relative to the unfertilized seedlings. There was an immediate pulse in nitrate levels following the first application of NH_4NO_3+K_2SO_4, but this was of short duration. Levels of most other nutrients continued to increase between September 16 and January 10, but these increases were generally unrelated to the fertilizer treatments. Although TKN levels increased, nutrient ratios determined as a proportion of TKN decreased, but generally remained within balance. Needle dry weights also increased at this time, but the increase was not related to the treatments applied. There were no differences in root growth potential (as measured by total new root dry weight) among the treatments. Seedlings that received 160 and 320 kg N/ha broke bud three days earlier than the unfertilized seedlings. Seedling cold hardiness LT_{50} levels on October 23, November

13, and December 9 showed no consistent significant differences among the fertilizer treatments. By December 30 all treatments had attained similar LT_{50} levels (-14 °C). Adding high levels of fertilizers after budset in the fall did not appear to disrupt the cold hardiness process. Detectable differences in baseline seedling variable chlorophyll fluorescence F_{var}/F_{max} levels among the treatments occurred on November 13 and December 30. Fertilized seedlings had consistently higher F_{var}/F_{max} than unfertilized seedlings. Fall fertilization to stimulate late season luxury consumption of nitrogen appears to be beneficial if conducted after seedlings have ceased growth and set bud. If no biologically significant nutrient imbalances occur as a result of fertilization, as was the case in this study (up to 320 kg N/ha), seedlings with elevated levels of nitrogen may outperform seedlings with lower levels of nitrogen.

Fall Fertilization Effects on Douglas-fir Seedling Quality

by

Todd M. Birchler

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Chapter 1

1.0 Introduction

The objective of forest tree nurseries is to produce seedlings that meet specified targets, i.e. those morphological and physiological characteristics that can be quantitatively linked with reforestation success (Rose et al. 1990). With limitations on vegetative control techniques it is increasingly important to reforest with quality seedlings capable of rapid establishment to provide rapid return on investment. Initial reforestation costs are high, but having to replant a site more than once may seriously decrease return on the investment. Standard grading practices which measure height and diameter to ensure the seedlings meet specified criteria are easy to do, but these measurements alone do not fully describe the physiological condition of seedlings and as such are not very useful for predicting outplanting performance in terms of survival and subsequent growth. Physiological characteristics such as seedling water status, mineral nutrition, carbohydrate contents, and cold hardiness are more difficult and time consuming to measure but provide more pertinent physiological information for predicting outplanting performance (Ritchie 1984, Rose et al. 1990).

Seedling mineral nutrition is an important consideration in producing quality seedlings and is commonly addressed through nursery fertilization programs. A fertilization regime of repeated and constant nutrient additions applied during the growing season is typical in bareroot nurseries. However, applying fertilizers when the seedlings are not actively growing may lead to additional uptake and possible improvement in nutrient reserves which may benefit the seedling after outplanting.

Fall fertilization practices may be a possible alternative for culturing seedlings with above normal levels of nutrition (nutrient loading). Fall fertilization regimes are easily implemented in both bareroot and containerized nurseries and are simply applied in

addition to conventional fertilization. The success of fall fertilization (and indeed any nursery cultural practice) can only be judged based upon physiological changes effected in the seedlings and the relationship of these changes to outplanting performance. The link between fall fertilization and outplanting performance is not direct. Therefore, evaluating fall fertilization requires an examination of some of the intermediate effects on seedling physiology such as nutrient levels, cold hardiness, root growth, bud break timing, and carbohydrate status. This study examines the effects of fall fertilization on Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco) physiology in the nursery, specifically nutrient dynamics, cold hardiness, variable chlorophyll fluorescence, root growth potential, and timing of bud break. Future studies involve the evaluation of the outplanting performance of the fall-fertilized seedlings.

Chapter 2 examines the effect of fall fertilization on nutrient concentrations, contents, and ratios over time. It also examines the effect of fall fertilization on root growth potential and timing of budbreak. Chapter 3 examines the effect of fall fertilization on seedling cold hardiness and variable chlorophyll fluorescence.

Chapter 2

2.0 Fall Fertilization Effects on Douglas-fir Seedling Nutrition in the Nursery

by

Todd M. Birchler

ABSTRACT. Coastal Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco) 1+1 seedlings were fertilized with two types of fertilizers (NH_4NO_3+K_2SO_4 and $(NH_4)_2SO_4$+KCl) at four rates (0, 80, 160, 320 kg N and K/ha) split over 3 application dates (September 19, October 13, November 1, 1996). By January 10, total Kjeldahl nitrogen (TKN) concentrations increased 16, 30, and 34% and contents increased 6, 20, and 26% for the 80, 160, and 320 kg N/ha treatments relative to the unfertilized seedlings. Potassium levels remained relatively unchanged as a result of the fertilization treatments. Chloride concentrations increased 57, 77, and 112% and contents increased 45, 71, and 92% for the 80, 160, 320 kg K/ha as KCl relative to the unfertilized seedlings. There was an immediate pulse in nitrate levels following the first application of NH_4NO_3+K_2SO_4, but this was of short duration. Levels of most other nutrients continued to increase between September 16 and January 10, but these increases were generally unrelated to the fertilizer treatments. Since TKN levels increased, nutrient ratios determined as a proportion of TKN decreased, but generally remained within balance. Needle dry weights also increased at this time, but the increase was not related to the treatments applied. There were no differences in root growth potential (as measured by total new root dry weight) among the treatments. Seedlings that received 160 and 320 kg N/ha broke bud a significant three days before the unfertilized seedlings. Fall fertilization to stimulate late season luxury consumption of nitrogen appears to be beneficial if conducted after seedlings have ceased growth and set bud. If no biologically significant nutrient imbalances occur as a result of this increase, outplanting performance of seedlings with higher levels of nitrogen may be enhanced.

2.1 Introduction

The objective of forest tree nurseries is to produce seedlings that meet specified targets, i.e. those morphological and physiological characteristics that can be quantitatively linked with reforestation success (Rose et al. 1990). With limitations on vegetative control techniques it is increasingly important to reforest with quality seedlings capable of rapid establishment in the midst of competing vegetation to provide rapid return on investment. Standard grading practices which measure height and diameter to ensure seedlings meet specified criteria are easy to do, but these measurements alone do not fully describe the physiological condition of seedlings and as such are not very useful for predicting outplanting performance in terms of survival and subsequent growth. Physiological characteristics such as seedling water status, mineral nutrition, carbohydrate contents, and cold hardiness are more difficult and time consuming to measure but provide more pertinent physiological information for predicting outplanting performance (Ritchie 1984, Rose et al. 1990).

Seedling mineral nutrition is an important consideration in producing quality seedlings and is commonly addressed through nursery fertilization programs. A survey of 19 Pacific Northwest nurseries reported the average amount of fertilizers applied per hectare each rotation included 224 kg nitrogen, 126 kg phosphorus, 103 kg potassium, 9 kg magnesium, 136 kg sulfur, and 557 kg ground limestone (Oregon State University Nursery Survey, Duryea and Landis 1984). These are typically applied as numerous topdressings during the growing season (van den Driessche 1984) with the objective of enabling the seedlings to attain desired morphological and physiological characteristics while maintaining nutrient levels within specific ranges. In a summary of October-collected foliar samples of 2+0 Douglas-fir (*Psuedotsuga menziesii* (Mirb.)Franco) grown in Pacific Northwest nurseries, analyses revealed adequate nutrient levels as follows: 1.8% nitrogen, 0.18% phosphorus, 0.8% potassium, 0.2 % calcium, 0.12% magnesium, 0.18% sulfur, 80 ppm sulfate, 390-1294 ppm manganese, 5.1-7.7 ppm copper, 9-39 ppm boron, and 17-63 ppm zinc (van den Driessche 1984). This conventional regime of repeated and

constant nutrient additions applied during the growing season is typical in bareroot nurseries.

Other nonconventional fertilization regimes (exponential, exponential loading, and late-season or fall fertilization) have the objective of producing seedlings with target morphology but with above normal levels of nutrition. Exponential fertilization provides the same quantity of nutrients as conventional regimes but the fertilizer is applied in levels that reflect the growth of the seedling. Smaller, more frequent doses gradually increase with the exponential growth of the seedling with the idea of constantly providing sufficient nutrients. The resulting seedling size is similar to conventionally fertilized seedlings, but nutrient levels are higher (Timmer and Armstrong 1987, Timmer et al. 1991, Miller and Timmer 1994, Timmer and Aidelbaum 1996). Exponential loading regimes apply supraoptimal quantities of nutrients as described above. This practice, depending on the species and amounts applied, may significantly alter seedling morphology (Miller et al. 1994). Depending on the objectives, this may not be desirable. In contrast, late-season fertilization provides additional nutrients to the soil after seedling growth has ceased, buds have set, and dormancy has been induced. With the cessation of shoot growth and the resumption of root growth (and continuation of caliper growth), any nutrient deficiencies incurred during the growing season may be addressed and luxury uptake (or nutrient loading) may be possible. As long as environmental conditions permit (i.e. adequate soil moisture and temperature), roots are still growing (i.e. occurrence of nonsuberized root tips), and nutrients are available in the rhizosphere, uptake should continue.

There are several impediments to the successful establishment of newly outplanted Douglas-fir seedlings. Immediate short-term survival depends upon the reestablishment of intimate root-soil contact and the reinitiation of water and nutrient uptake (Burdett 1990). Under favorable conditions for photosynthesis, new root growth in Douglas-fir utilizes energy derived from current photosynthate (van den Driessche 1987). Where conditions do not permit adequate photosynthesis, new root growth may rely on stored photosynthate (Olofinboba and Kozlowski 1973). Photosynthesis requires not only adequate moisture but also nutrients, especially nitrogen. Nitrogen is required in relatively large quantities and is typically the most limiting nutrient at the outplanting site. Until new

root growth occurs, newly planted seedlings rely on nutrient retranslocation from within the plant to satisfy the needs for photosynthesis, other physiological processes, and perhaps shoot growth (Krueger 1967). Long-term survival and growth depends upon continued exploitation of the soil profile and ability to compete with adjacent vegetation for resources. Therefore, it appears that seedlings containing balanced nutrition at higher levels (nutrient reserves) may be more competitive and successful than similar sized seedlings with lower nutrient levels.

Exponential fertilization and fall fertilization practices are two possible alternatives for culturing seedlings with above normal levels of nutrition (nutrient loading). Exponential fertilization regimes have been commonly utilized in and are best suited to container nurseries. Fall fertilization regimes are easily implemented in both bareroot and containerized nurseries and are simply applied in addition to the conventional fertilization. The success of fall fertilization (and indeed any nursery cultural practice) can only be judged based upon its relationship with outplanting performance. The link between fall fertilization and outplanting performance is not direct. Therefore, evaluating fall fertilization requires an examination of some of the intermediate effects on seedling physiology such as nutrient levels, cold hardiness, root growth, bud break timing, and carbohydrate status. These parameters aid in understanding why seedlings performed the way they did.

This study examines the effects of fall fertilization on Douglas-fir seedling physiology in the nursery, specifically nutrient dynamics, root growth potential, and bud break timing. Since an evaluation of this process would not be complete without attempting to link fall fertilization with outplanting performance, a future study will evaluate the field survival and growth of fall-fertilized seedlings.

2.1.1 Literature Review

Fall fertilization studies initiated in the 1920's and 1930's examined the effect of fall applied nitrogen on uptake, growth, and vigor of apple and peach trees (Hooker 1922, Aldrich 1931, Weinberger and Cullinan 1934, Smith 1935, Batjer et al. 1939, Batjer et al. 1943). Fall applied nitrogen was taken up (Smith 1935), stored in the roots, then

translocated to the terminals the following year in apple trees (Aldrich 1931), but there was little difference in subsequent growth of fall- or spring-fertilized peach trees (Weinberger and Cullinan 1934). Studies on forest seedlings were of interest briefly in the 1950's and consisted of evaluating the survival and growth of seedlings fertilized in the fall with nitrogen and potassium prior to lifting and outplanting (Ursic 1956, Gilmore et al. 1959, Shoulders 1959). Fall fertilization studies were reinitiated in the 1970's and several studies were conducted on Douglas-fir during the 1980's (Benzian et al. 1974, van den Driessche 1985, van den Driessche 1988, Margolis and Waring 1986).

A description of the results of previous fall fertilization studies on tree seedlings must be general due to variations in fertilizer types, rates, and forms utilized, the number of applications, initial nitrogen levels, and analysis dates. Increases in Douglas-fir foliar nitrogen concentrations ranged from 15 to 70% as a result of fall fertilizer applications (Simpson 1985, van den Driessche 1985, Margolis and Waring 1986, Brown 1988, van den Driessche 1988). Increases in foliar phosphorus levels ranged from 11 to 55% (Simpson 1985, van den Driessche 1985, van den Driessche 1988), but changes in potassium concentrations were infrequent, ranging from a 16% increase (Simpson 1985) to a 27% decline (van den Driessche 1988). The balance of micronutrients is as important to seedling physiology and subsequent outplanting performance as are the macronutrients, but no mention is made of the effects of fall fertilization on micronutrient levels.

Increases in seedling nitrogen levels as a result of fall fertilization with nitrogen and potassium were also reported by Benzian et al. (1974) for Sitka spruce (*Picea sitchensis* (Bong.) Carr.), Norway spruce (*Picea abies* (L.) Karsten), lodgepole pine (*Pinus contorta* Dougl.), western hemlock (*Tsuga heterophylla* (Rafn.) Sarg.), and grand fir (*Abies grandis* (Dougl.) Lindl.) and by Hinesley and Maki (1980) for longleaf pine (*Pinus palustris* Mill.). Potassium levels were largely unaffected.

Increases in foliar nutrient levels as a result of fall fertilization may have several implications. Increased foliar nitrogen concentrations resulted in increased free amino acid concentrations in Douglas-fir seedlings during the winter which can then be utilized for protein synthesis (Margolis and Waring 1986). However, slight declines in root total nonstructural carbohydrates resulted.

The effect of increased seedling nutrients on cold hardiness has been variable. Fall-applied nitrogen, or nitrogen with phosphorus, increased seedling cold hardiness in Douglas-fir, but phosphorus applied without nitrogen decreased seedling cold hardiness (Thompson 1983). Nutrient concentrations were not provided. Ponderosa pine (*Pinus ponderosa* Laws) cold hardiness also improved with increasing nitrogen concentrations (Gleason et al. 1990). Both of the above studies tested cold hardiness only once, utilizing the whole-plant freeze test, but description of the subsequent determination of seedling vitality was not clear.

Root growth potential significantly increased in fall-fertilized as opposed to conventionally fertilized Douglas-fir (van den Driessche 1988). Compared to unfertilized controls, root growth was greatest with the 85 and 170 kg N/ha fall fertilization treatments (Simpson 1985). Bud break timing was generally faster in fall-fertilized Douglas-fir than unfertilized (Thompson 1983, van den Driessche 1985, Margolis and Waring 1986). Earlier bud break effectively lengthens the growing season which may result in increased growth. However, if bud break is followed by a late spring frost, then seedling growth and vigor may be compromised.

If fall fertilization can lead to an increase in seedling nitrogen levels (luxury consumption), and other possible benefits such as improved cold hardiness, root growth potential, and bud break timing, then why is it not a common nursery cultural practice? Perhaps it is because there is insufficient or inconsistent evidence that links fall fertilization and elevated nutrition (especially nitrogen) with outplanting performance and therefore the increased cost in the nursery is difficult to justify.

Due to outplanting site microclimate variability and other interrelating factors such as seedling water and carbohydrate status and seedling handling, the relationship between fall fertilization, seedling nutrient levels, and outplanting performance has been variable. September-fertilized 2+0 Douglas-fir with 57.5 kg N/ha resulted in an increased second-year survival of 7% and a greater average yearly leader growth after outplanting. This was maintained throughout the succeeding five years (Anderson and Gessel 1966). Fall-fertilized Douglas-fir seedlings had significantly greater survival for the first two years than conventionally fertilized seedlings (5.7 and 6.6%) and also exhibited greater height

and diameter growth, and height and volume relative growth rates (van den Driessche 1988). Survival related curvalinearly with seedling nitrogen concentration with the highest survival related to intermediate (2.1%) nitrogen levels (r^2=0.44). Applying nitrogen, phosphorus, and potassium during the hardening off period of containerized Douglas-fir resulted in increases in nitrogen and phosphorus, shoot growth and root growth after one year in the field (Jopson and Paul 1984). Douglas-fir height growth after outplanting was not affected by fall fertilization with nitrogen, but stem caliper was increased (Brown 1990). Shoot growth, leaf area, relative growth rate, and production per unit nitrogen was greater in fall-fertilized Douglas-fir seedlings after outplanting (Margolis and Waring 1986). Total nitrogen levels were higher as well due to the increased seedling size.

2.1.2 Objectives and Null Hypotheses

The objective of this study was to determine the effects of fall fertilization on seedling nutrient levels, root growth potential, and bud break timing. This study incorporated fertilizer types and fertilizer rates which were expected to result in significant nutrient uptake. Three fertilizer applications and five nutrient analysis dates were utilized to better understand Douglas-fir seedling nutrient dynamics during the fall and winter prior to lifting and outplanting. The effects of fall fertilization on seedling needle dry weights were examined. Apparent nitrogen recovery was determined to evaluate the efficacy of the various rates of nitrogen applied.

Null hypotheses tested were: Fall fertilization with NH_4NO_3+K_2SO_4 or $(NH_4)_2SO_4$+KCl does not affect:

1. concentrations and contents of N, K, Cl, NO_3, SO_4, P, Ca, Mg, and micronutrients
2. root TKN concentrations
3. nutrient ratios
4. needle dry weights

at each harvest date and over time between September and January, and fall fertilization does not affect seedling:

5. root growth potential
6. bud break timing

2.2 Materials and Methods

2.2.1 Nursery Stock

Two year old (1+1) bareroot Douglas-fir seedlings from a coastal Oregon seed source (M417295 071C12, elev. 1500 ft) were grown under standard nursery cultural practices at the D.L. Phipps State Forest Nursery located 5 km south of Elkton, Oregon. Seedlings were sown in March 1995, lifted, pruned, and transplanted during October 1995 at a density of approximately 12.5 seedlings per linear meter (74 seedlings per square meter). After transplanting, but before the initiation of this study, the seedlings had received approximately 126 kg N/ha, 8 kg P/ha, 15 kg K/ha, and 27 kg S/ha along with micronutrients applied over four applications between late March and early June. Seedlings were topmown in July, wrenched in early September, and drought stressed for 2 weeks prior to the initiation of this study to induce bud set and the onset of dormancy. Treatments for this study began on September 19, 1996.

2.2.2 Fertilizer Application

The treatments consisted of two types of fertilizers (NH_4NO_3+K_2SO_4 and $(NH_4)_2SO_4$+KCl) at four rates (0, 80, 160, 320 kg/ha) of total nitrogen and potassium

divided over three application dates (i.e. the first application for the 80 kg/ha rate would be 80/3 or 26.7 kg/ha). Three equal applications were implemented to maximize the duration of the nutrients in the rooting zone since all of the included cations and anions are potentially leachable from the soil profile and typical fall and winter precipitation in the Pacific Northwest can be quite high. Table 1 includes all of the nutrients added with these fall applications. Determinations of the amount of bulk fertilizer to apply to achieve the above rates of nitrogen and potassium are included in Appendix Table I along with the total amounts of all nutrients supplied during the applications.

TABLE 1. Total amounts of nutrients applied (kg/ha).

Fertilizer Type	Rate	NH4	NO3	N	K	SO4	S	Cl
NH4NO3+K2SO4	80	51.4	177.1	80.0	80.0	98.2	32.8	0.0
	160	102.9	354.3	160.0	160.0	196.4	65.6	0.0
	320	205.7	708.6	320.0	320.0	392.7	131.1	0.0
(NH4)2SO4+KCl	80	103.0	0.0	80.0	80.0	207.9	69.4	72.7
	160	206.0	0.0	160.0	160.0	415.9	138.9	145.3
	320	412.0	0.0	320.0	320.0	831.7	277.7	290.7

Fertilizers were applied on September 19, October 11, and November 1 using a CO_2 powered applicator constructed of 1/2 inch PVC tubing. The applicator had six nozzles designed to apply fertilizers between the rows of seedlings as close to the ground as possible. Due to varying distance of each nozzle from the pressure source, differing spray velocities emitted by the nozzles were corrected by utilizing nozzles of three different apertures to achieve relatively uniform spray quantities. This was determined by spraying the nozzles into containers to quantify the volume. The fertilizers were added to water in a three gallon tank to allow for good dissolution of the fertilizers and sufficient multiple passes by the applicators to ensure uniform application. Premixed concentrated solutions were filtered into the spray tank and any resulting residue was redissolved two to three times to ensure the maximum dissolution of the fertilizers.

2.2.3 Seedling Sampling

Seedlings were sampled from each treatment plot for both nutrient analyses and cold hardiness using a randomly predetermined sampling format (Figures I and II in appendix). Samples were harvested on September 16, October 8, November 1, November 22, 1996 and January 10, 1997. A shovel was used to cut around all sides of the group of sample seedlings and the seedlings were carefully lifted from the seedbed. The seedlings were washed free of soil, placed in coolers, and transported to Oregon State University. Six to ten seedlings were harvested from each treatment plot on each date.

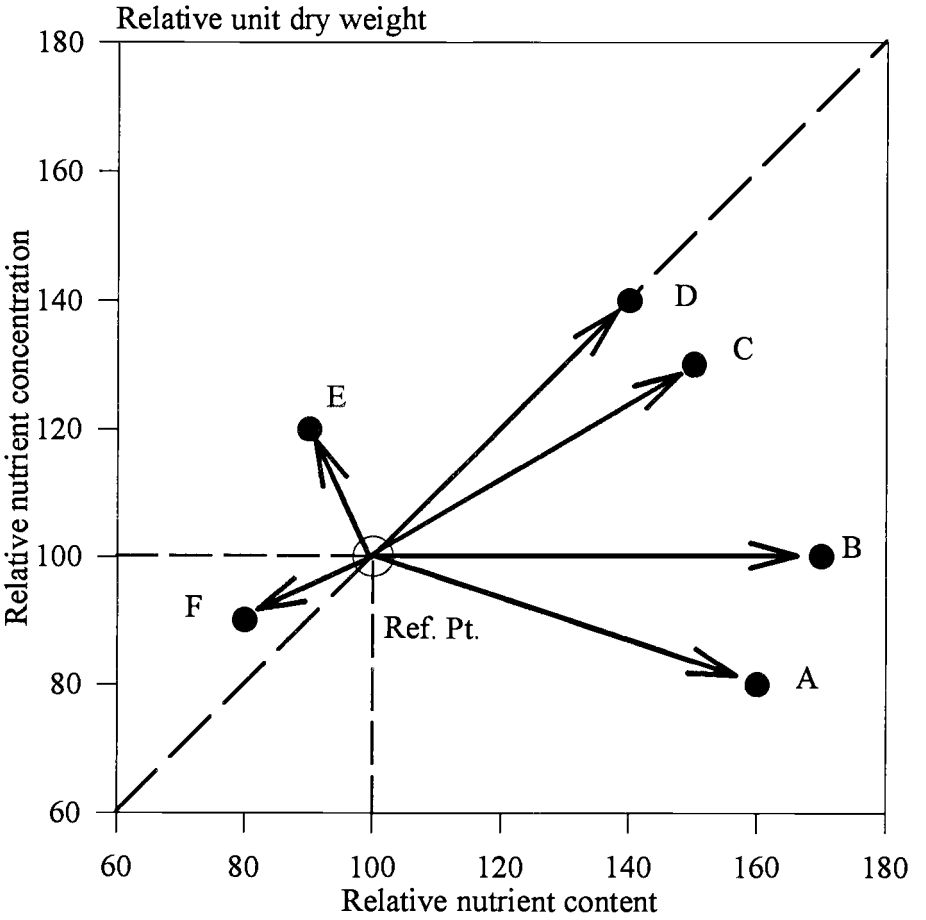
Seedlings were severed at the cotyledon scar, separated into roots and shoots, and dried for 72 hours at 70° C. Needles were removed from the shoots. Both foliage and roots were ground with a Wiley mill through a #40 mesh screen. Needles were submitted for total Kjeldahl nitrogen analysis (TKN), inductively coupled argon plasma analysis (ICP), and ion chromatography. A portion of the roots were submitted for TKN analysis and the remainder placed into a freezer at -20° C to await possible starch analysis. The total number of needles per seedling were quantified using a sample of 10 seedlings so estimations of apparent nitrogen uptake could be determined (see section 2.2.7). A sample of 100 needles from each treatment replicate were 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 fertilization effects (Timmer and Stone 1978, Haase and Rose 1995). A reference point is chosen (the unfertilized treatment in this study) and set to 100. Subsequent data points are 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 1). The vector diagrams presented in this study compare nutrient shifts relative to two different reference points. The first normalizes the unfertilized control treatments to 100 and all nutrient shifts resulting from the different fertilizer rates are compared relative to the controls for a particular harvest date. The second normalizes the initial September nutrient levels to 100 and shifts of each respective nutrient on

subsequent dates are compared relative to their initial level to examine nutrient dynamics over time for each fertilizer rate. In addition, the ratio of nutrient levels over time relative to nitrogen were examined to determine whether the treatments led to the creation of a nutrient imbalance. Foliar TKN concentrations were set to 100 and nutrient ratios were determined as a proportion of the TKN concentration.

2.2.4 Nutrient Analyses

MicroKjeldahl: Total Kjeldahl nitrogen (TKN) determination used the microKjeldahl method adapted from Gavlak et al. (1994). Approximately 0.25 grams of foliar (or root) sample was weighed into Kjeldahl tubes to which a K_2SO_4 + $CuSO_4$ + Se accelerator and 8 ml of concentrated sulfuric acid were added. After mixing with a vortex mixer, the samples were then placed in digestion blocks. The digestion consisted of two steps: a low temperature (150° C) and high temperature (350° C) digestion. After cooling, the samples were then diluted and brought to volume with deionized water and inverted several times to mix the solution. Then, a portion of the solution was placed into the sample vials and submitted for autoanalysis. This analysis determines percent total nitrogen which includes all forms of nitrogen except for the nitrate fraction, which is not completely determined through this method (some nitrate is volatilized during the digestion process).

Inductively Coupled Argon Plasma Spectrometry (ICP) (Thompson and Walsh 1989): The nutrient extraction procedure was adapted from Gavlak et al. (1994). Approximately 0.50 g of foliar sample was placed in quartz test tubes in a high temperature oven for dry ashing (approximately 24 hours). Dilute nitric acid was then added for cation extraction and left overnight. Concentrations of P, K, Ca, Mg, Mn, Fe, Cu, Zn, and B were determined using a Perkin Elmer Optima 3000DV ICP Emission Spectrometer (Norwalk, CT) interfaced with ICP WinLab version 1.06.



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 1. Interpretation of directional shifts in nutrient concentratration, content, and dry weight. Adapted from Timmer and Stone (1978).

Ion Chromatography: Twenty ml of deionized water were added to approximately 0.20 gram of foliar sample. This was shaken for one hour then filtered through #42 Whatman filter paper (Ellen Bush, Oregon State University Central Analytical Laboratory, personal communication). One ml of sample was then diluted with 3 ml of deionized water and submitted for analysis. A Dionex System 2000i (Sunnyvale, CA) containing a Dionex AS4a-SC column was utilized. This system was interfaced with AutoIon 400 Chromatography Software (Dionex). Anions determined (ppm) included NO_3, SO_4, HPO_4, and Cl. Results from the ion chromatograph were then adjusted for the dilution and sample weights used.

2.2.5 Nursery Soil and Climate Data

Prior to the first fertilizer application and two weeks after the final fertilizer application (September 19 and November 21, 1996) two soil samples were taken from each treatment plot in block 2. Using a spade, soil was sampled to a depth of approximately six inches and mixed in a bucket. A composite sample was submitted for analysis at the Central Analytical Laboratory at Oregon State University. The soils were dried, ground, and analyzed for pH, N, S, P, K, Ca, Mg, Mn, Fe, B, Cu, and Zn (Horneck et al. 1989) (Table II in appendix).

Minimum and maximum air temperatures and precipitation were recorded daily at Phipps Nursery from September through January. Due to equipment malfunction, the attempt to record soil temperatures in block 2 using a Telog auto recorder failed, although nursery data was available for December and January. Nursery climate data is included in the appendix (Figure III in the appendix).

2.2.6 Root Growth Potential and Bud Break Timing

A study to examine root growth potential and bud break timing was initiated on January 24. A sample of 12 seedlings from each treatment plot were lifted on January 10 and stored in the cooler

for two weeks. Twelve aquariums (four blocks of three aquariums, each aquarium contained one seedling from each treatment) with aerating wands were placed in a greenhouse with a 13 hour photoperiod and air temperatures between 15.5 and 26.7° C. Relative humidity was not controlled. The water temperature in the aquariums ranged from 16.7 to 20.0° C. Before seedlings were placed into the aquariums, all white root tips were removed. Beginning at day 20, seedlings were examined at two to three day intervals for evidence of bud break. After 32 days, seedlings were removed and all new root growth (greater than 1 cm in length) was removed, dabbed dry, and weighed.

2.2.7 Apparent Nitrogen Recovery

It is important to determine which rate of nitrogen application resulted in the most efficient uptake by the final harvest date so that fertilizer waste is limited. The difference in uptake between the unfertilized seedlings and those that received 80 to 320 kg N/ha is expanded to kg N/ha and the apparent nitrogen recovery is determined as a proportion of the nitrogen applied. Although not commonly done with tree seedling fertilization programs, this is a common method in agronomic studies (Mengel and Kirkby 1987):

$$\text{Apparent N recovery} = \frac{\text{N uptake (fertilized)} - \text{N uptake (control)}}{\text{N fertilizer applied}} \times 100\%$$

This is only a gross estimate which can be used to make relative comparisons among the rates of nitrogen applied. Since only the foliage is considered, this should not be mistaken for actual recovery. It is a gross estimate because of the variability involved in expanding nitrogen concentration as a percent of dry weight to kg N/ha:

$$\%N \times g/needle \times needles/seedling \times 73.5\ seedlings/m^2 = g\ N\ taken\ up/m^2$$

$$g\ N\ taken\ up/m^2 * 1kg/1000g * 10000m^2/ha = kg\ N/ha$$

The number of needles per seedling was estimated from 10 seedlings and the number of seedlings/m^2 was estimated from ten linear meter counts multiplied by the nursery bed

width (1.2 m). Thus, only the TKN concentration and dried needle weights varied among the fertilizer rates (the values used in this instance were the means of the four replicates). These considerations notwithstanding, the apparent nitrogen recovery in the foliage of the seedlings serves as a comparison only for the determination of whether there was a point of diminishing returns given the amounts of nitrogen applied in this study. This is an important consideration for the design of future studies and for the implementation of a fall fertilization program.

2.3 Experimental Design and Statistical Analysis

The experimental design was a randomized complete block design with four blocks and a 2x4 factorial (two fertilizer types and four rates). The eight treatments were randomly assigned to a 6 m length of nursery bed on each of four nursery beds (blocks or replications, see appendix).

Nutrient and dry weight data were analyzed on each harvest date using analysis of variance (ANOVA) to examine the effects of the fertilizer types and rates on needle weights, and nutrient concentrations, contents, and ratios. At each harvest date, Fisher's Protected Least Significant Difference (FPLSD) procedure was utilized to determine significant differences among means (Steel and Torrie 1980). The assumptions of normality, linearity, and constant variance were verified by examination of the residuals and no transformations were necessary. The ANOVA table is included below.

ANOVA Table:

Source	df	E(MS)	F-test
Total	31		
Block (B)	3	$\sigma_\varepsilon^2 + \kappa' \sigma_B^2$	MSE_B/MSE_ε
Treatment (A)	7	$\sigma_\varepsilon^2 + \kappa\varphi_T^2$	MSE_T/MSE_ε
Type	(1)		
Rate	(3)		
Type x Rate	(3)		
Error	21	σ_ε^2	

Needle dry weight, and nutrient concentration and content trends over time by treatment were examined as repeated measures analysis of variance utilizing multivariate analysis of variance (MANOVA). The effects of time, and interactions of fertilizer type and fertilizer rate with time were examined using Wilks' Lambda criteria (Johnson and Wichern 1988).

Statistical Analysis Software (SAS Institute 1989) was used for all data analysis. Tables of P-values for each of the nutrient concentrations, contents, and ratios as well as mean squares for nutrient concentrations are included in the appendix (Tables III to V). P-values for the effect of time and time by treatment interactions are also included.

2.4 Results

2.4.1 General Results

Fall fertilization with NH_4NO_3+K_2SO_4 and $(NH_4)_2SO_4$+KCl led to significant increases in foliar TKN concentrations and contents, root TKN concentrations, foliar Cl concentrations and contents, and a temporarily significant increase in NO_3 concentrations compared to the unfertilized seedlings. Potassium levels changed slightly over time, but were unaffected by the treatments. The levels of P, Ca, Mg, Mn, Fe, Cu, B, and Zn were generally not affected by fertilization. January nutrient ratios did not become imbalanced when compared to the initial September levels (with the possible exception of the N/K ratio) even though nitrogen concentrations increased nearly 30%. Needle dry weights increased over time, but this increase was not statistically related to the treatments. Seedling root growth potential was not affected by fall fertilization-induced increases in nitrogen. Bud break was significantly earlier in seedlings fertilized with 160 and 320 kg N/ha. All nutrient contents except Fe increased between September and January. Nitrogen appeared to be limiting, but did not hinder the continued increases in foliar levels of other nutrients.

2.4.2 Foliar Total Kjeldahl Nitrogen

There were no fertilizer type effects on mean foliar TKN concentrations and contents on any of the dates. Foliar TKN concentrations of unfertilized seedlings increased to November 22 before declining slightly. Large differences existed among the fertilizer rates by January 10 and the differences directly corresponded to the rate of nitrogen applied.

Mean foliar TKN concentrations differed significantly by fertilizer rate on November 1, November 22, and January 10 (P=0.0006, 0.0003, 0.0001). Seedlings fertilized with 320 kg N/ha had a significantly higher TKN level (2.17%) than seedlings fertilized with 80 kg N/ha (1.99%) which was significantly greater than the unfertilized seedlings (1.80%, Figure 2). These levels remained stable through November 22. On January 10, differences in TKN levels among the four fertilizer rates were even greater (1.67, 1.94, 2.17, 2.24%, respectively). A vector diagram using the unfertilized seedlings as a reference point set to 100 (Figure 3) illustrates the differences among treatments on four harvest dates. Foliar TKN of unfertilized seedlings increased from 1.56% to 1.81% by November 22 before declining to 1.67% in January.

Mean foliar TKN contents differed significantly on November 22 and January 10 (P=0.0009,0.0224). Fertilized seedlings had significantly more nitrogen (10.6, 10.5, 11.5 mg/100 needles) than unfertilized seedlings (8.9 mg/100 needles) on November 22 (Figure 2). By January 10, the foliar TKN contents of the 160 and 320 kg N/ha treatments were significantly higher (11.2, 11.7 mg/100 needles) than the unfertilized seedlings (9.3 mg/100 needles). Foliar TKN contents of unfertilized seedlings increased from 6.5 to 9.3 mg/100 needles between September and January.

There was a significant rate by time interaction effect on foliar TKN concentrations and contents as suggested by the data in Figure 2 (P=0.0016, 0.0039). The greatest increase in foliar TKN concentrations and contents between September and January occurred for the 160 and 320 kg N/ha treatments (from approximately 1.6 to 2.49% and 6.6 to 11.4 mg/100 needles, respectively). Unfertilized seedling foliar TKN concentration initially increased from 1.56 to 1.81% by November 22, but decreased to 1.67% by January. Contents initially increased to 9.03 mg/100 needles on November 1, then remained stable.

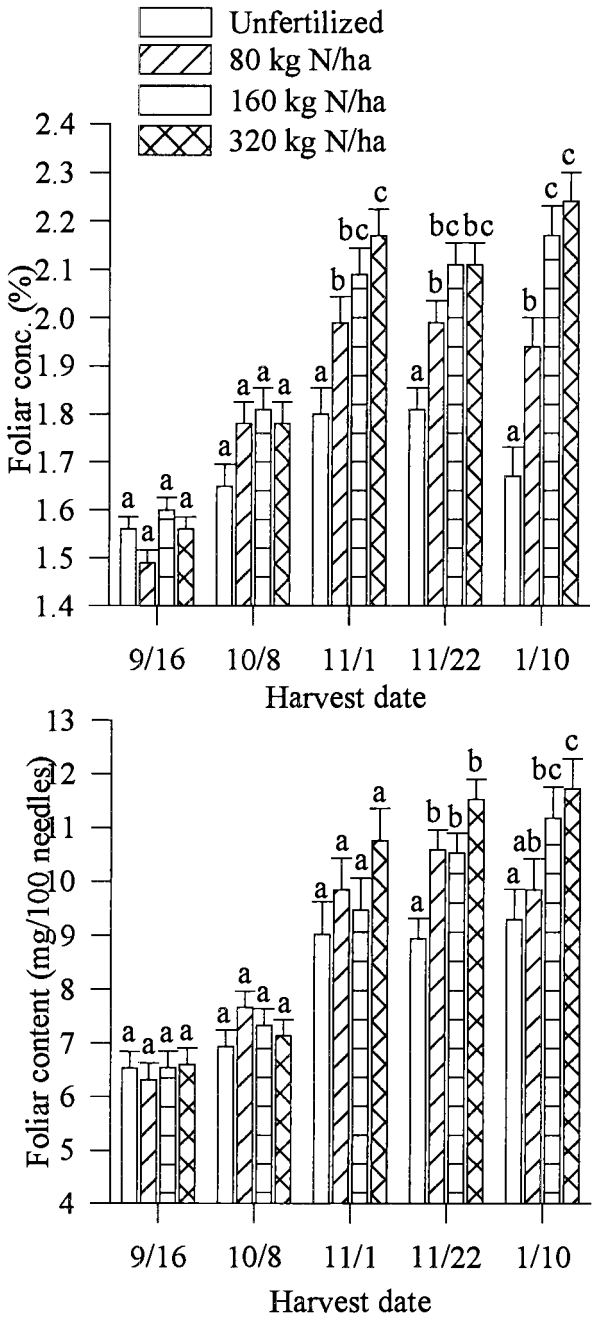


FIGURE 2. Foliar TKN concentration and content by fertilizer rate. Means followed by different letters within a date were significant at P=0.05.

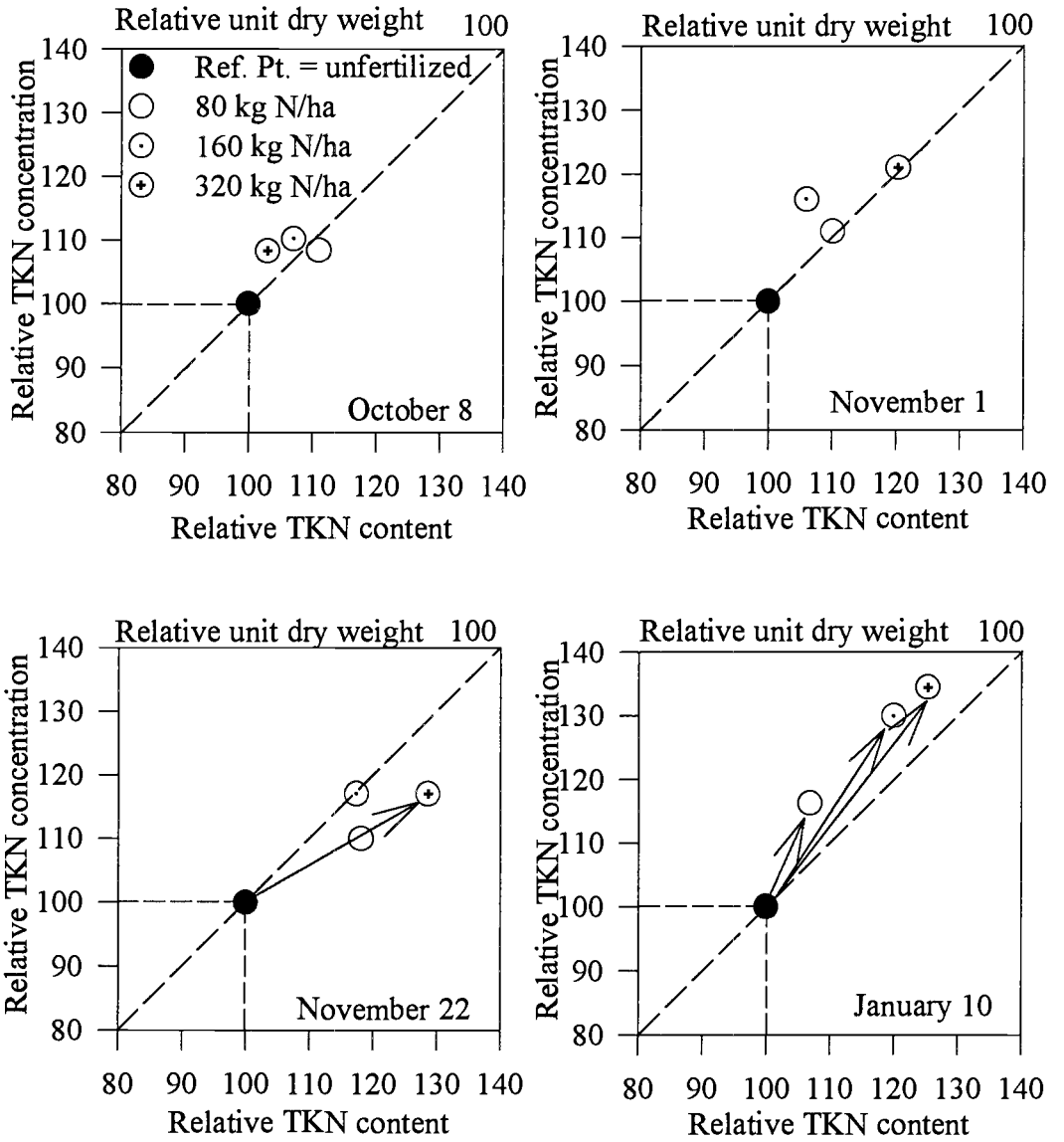


FIGURE 3. Shifts in relative TKN levels over time by fertilizer rate. The reference point is the TKN level of the unfertilized control at each respective date set to 100. TKN levels among the unfertilized, 80, and 160 kg N/ha treatments were significantly different on January 10 (P=0.05).

Vector diagrams (Figure 4) show the relative changes in foliar TKN levels by fertilizer rate over time. Each rate is compared to its initial September level (the reference point) set to 100. Small, nonsignificant, increases were evident immediately. By January, large differences existed among the unfertilized, 80, and 160 kg N/ha treatments.

Nutrient ratios fluctuated widely during the course of the study due to the significant decrease in foliar TKN levels on November 1 followed by the significant increase on January 10. Since the most current nutrient levels immediately prior to outplanting are of the greatest interest, only those ratios are presented (Table 2, see Table Vc for the ratios from each harvest date). On January 10, all nutrient ratios examined differed significantly by fertilizer rate except for sulfate and B. In all instances the nutrient ratios of the unfertilized treatment were significantly different from the 320 kg N/ha treatment.

2.4.3 Root Total Kjeldahl Nitrogen

Mean root TKN concentrations did not fluctuate as greatly as foliar TKN levels between September and January. There were no fertilizer type effects. Significant differences among fertilizer rates occurred sooner, and differences among rates were greater by January, than was observed in the foliage. Root TKN concentrations initially decreased, but each treatment increased on subsequent harvests.

Mean root TKN levels differed significantly by fertilizer rate on October 8, November 1, November 22, and January 10 (P=0.0065, 0.0081, 0.0016, 0.0001). On October 8, the unfertilized seedlings had less TKN (0.71%) than the fertilized seedlings (0.79, 0.78, 0.82%) (Figure 5). This difference continued through November 1 and 22. On January 10, differences in TKN due to fertilizer rate were even greater (0.86, 1.08, 1.28, 1.41%, respectively). Root TKN concentrations of unfertilized seedlings declined from 0.97% to 0.86% from September 16 to January 10.

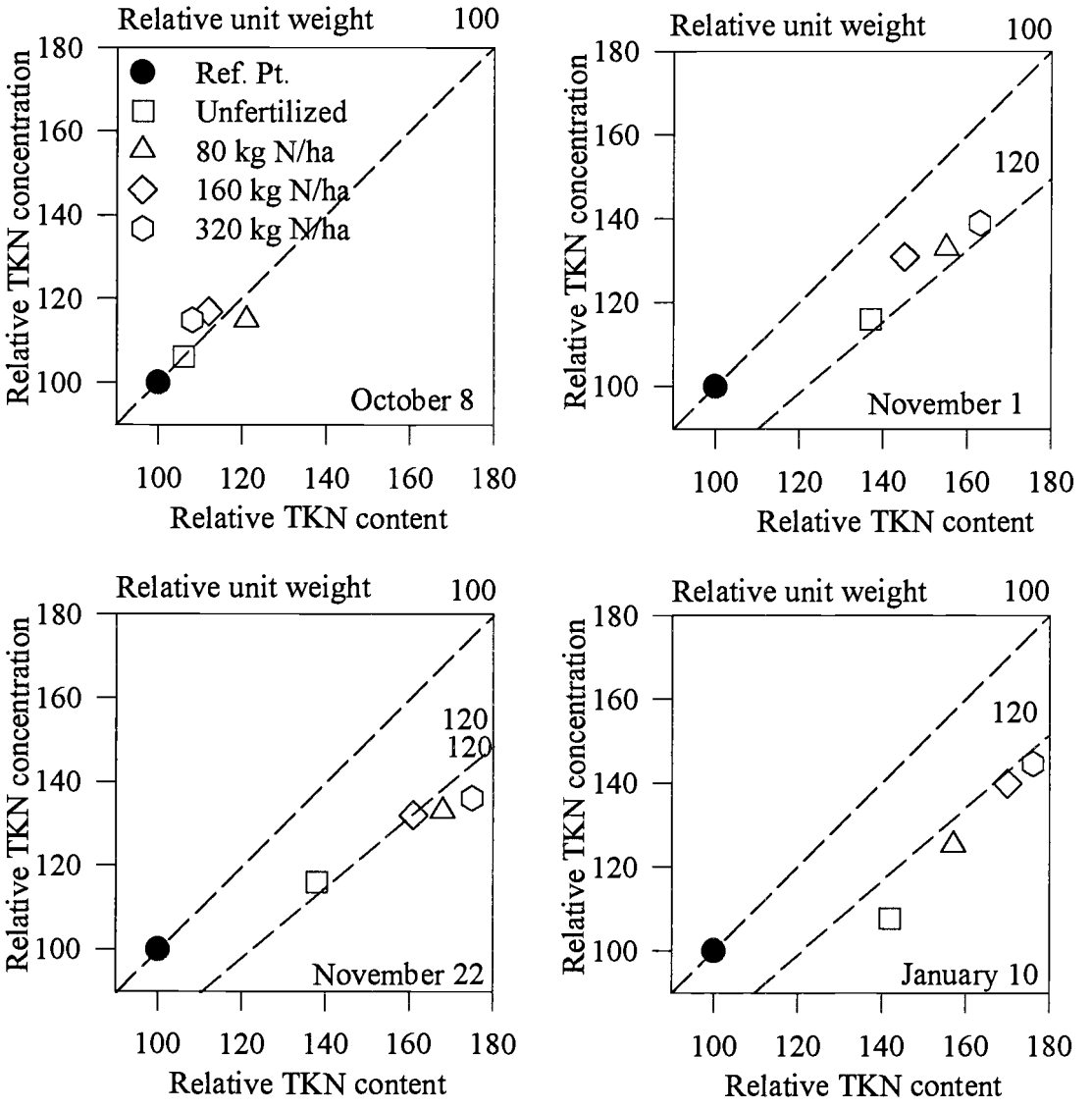


FIGURE 4. Relative TKN shifts over time by fertilizer rate. The reference point is the September TKN level for each respective nutrient set to 100. TKN levels among the unfertilized, 80, and 160 kg N/ha were significantly different on January 10 (P=0.05).

TABLE 2. January 10 nutrient ratios by fertilizer rate.

Rate	N	P	K	Ca	Mg	Mn	Fe	Cu	B	Zn*	SO4	S
0 kg N/ha	100	10.8b	51.4b	23.6c	7.6c	1.2b	1.68b	0.03b	0.13	0.20b	11.24	
80 kg N/ha	100	9.8b	47.5b	20.8b	6.8b	1.0ab	1.67b	0.03b	0.13	0.20b	10.84	
160 kg N/ha	100	8.1a	40.7a	19.0ab	6.1a	0.9a	1.35a	0.02a	0.11	0.14a	10.00	
320 kg N/ha	100	8.3a	39.0a	17.9a	5.8a	0.8a	1.30a	0.02a	0.10	0.14a	10.11	
Krueger (low) a	100	8.2	34.2	16.8	5.5	1.28	0.59	0.03	0.01	0.18		
Krueger (high) b	100	19.0	70.4	27.4	7.1	4.30	1.12	0.07	0.04	0.26		
Krueger c	100	15.7	46.1	17.3	6.6	2.24	0.43	0.03	0.04	0.18		9.9
van den Driessche d	100	10.0	44.4	11.1	6.6	2.2-7.2		0.03-0.04	0.05-0.22	0.09-0.63	0.44	10.0

* there was a fertilizer type by fertilizer rate interaction on this date for Zn
a lower range of ratios for fall harvested 2+0 nursery grown Douglas-fir seedlings (Krueger 1967)
b upper range of ratios for fall harvested 2+0 nursery grown Douglas-fir seedlings (Krueger 1967)
c values for 3 to 5 year old forest grown Douglas-fir seedlings (Krueger 1967)
d summary of extrapolated values for October harvested 2+0 Douglas-fir (van den Driessche 1984)

There was a significant rate by time interaction effect on mean root TKN concentrations (P=0.0003). Between September 16 and October 8, root TKN concentrations of seedlings fertilized with 320 kg N/ha decreased the least (from 0.92 to 0.82%) and unfertilized seedlings decreased the most (0.97 to 0.71%, Figure 5). Similarly, between November 22 to January 10, root TKN concentrations of seedlings fertilized with 320 kg N/ha increased the most (from 1.19 to 1.41%) whereas unfertilized seedlings increased least (0.84 to 0.86%).

2.4.4 Seedling Needle Weights

There were no treatment effects on mean weight per 100 oven dried needles on each harvest date (Figure 6). Dried needle weights varied considerably which made detecting treatment differences difficult. Mean levels by harvest date significantly increased over time from a mean of 0.42 g/100 needles in September to 0.53 g/100 needles in January (P=0.0001). Interestingly in January, the unfertilized seedlings had the largest needle weights!

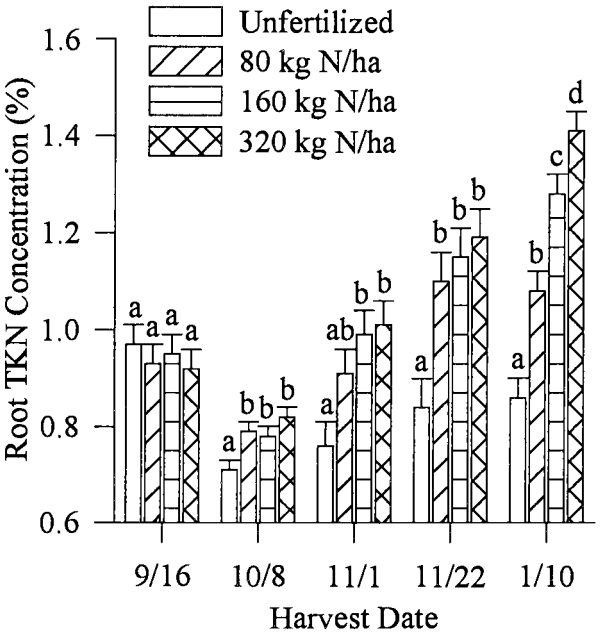


FIGURE 5. Root TKN concentrations over time by fertilizer rate. Means followed by different letters within a date were significant at P=0.05.

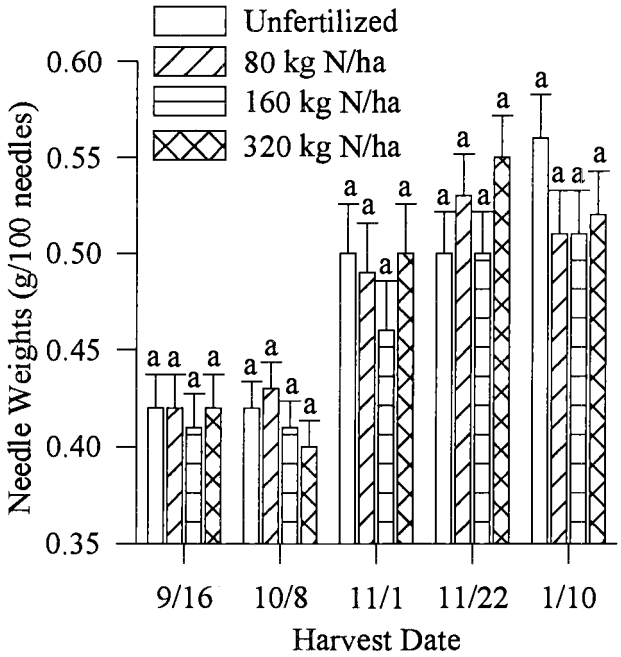


FIGURE 6. Needle weights over time by fertilizer rate. Means followed by different letters within a date were significant at P=0.05.

2.4.5 Nitrate

As expected, seedling foliar nitrate levels were temporarily increased as a result of applying NH_4NO_3 (177, 354, 709 kg NO_3/ha). Seedlings fertilized with NH_4NO_3 had higher levels of nitrate on all harvest dates. However, there were no differences in overall TKN levels. The pattern of foliar nitrate levels over time was similar to that of TKN.

Initial foliar nitrate levels were approximately 200 ppm. On October 8, after 1/3 of the fertilizers had been applied, seedlings treated with 80, 160, and 320 kg N/ha as NH_4NO_3 had significantly greater foliar nitrate levels (241, 281, 355 ppm, Figure 7) than those unfertilized (214 ppm) and the observed levels corresponded to the rate of NH_4NO_3 applied (P=0.0001). On November 1, this same relationship continued (P=0.0038), but at a much decreased concentration of nitrate (approximately 175 to 200 ppm). By November 22 and January 10, there were no significant differences among the rates of the NH_4NO_3 applied, but seedlings fertilized with NH_4NO_3 contained significantly higher levels of nitrate (190 and 252 ppm) than seedlings fertilized with $(NH_4)_2SO_4$ (185 and 247 ppm) (P=0.0485, 0.0222).

On October 8, seedlings fertilized with NH_4NO_3 had significantly higher nitrate contents (0.11, 0.11, 0.14 mg/100 needles) than unfertilized seedlings or seedlings fertilized with $(NH_4)_2SO_4$ (0.8 to 0.9 mg/100 needles, Figure 7, $(NH_4)_2SO_4$-fertilized seedlings not shown) (P=0.0001). By January 10, all seedlings had similar quantities of nitrate (0.12 to 0.14 mg/100 needles). A vector diagram (Figure 8) illustrates the similarities among the fertilizer rates of nitrate-fertilized seedlings on January 10 relative to the unfertilized controls.

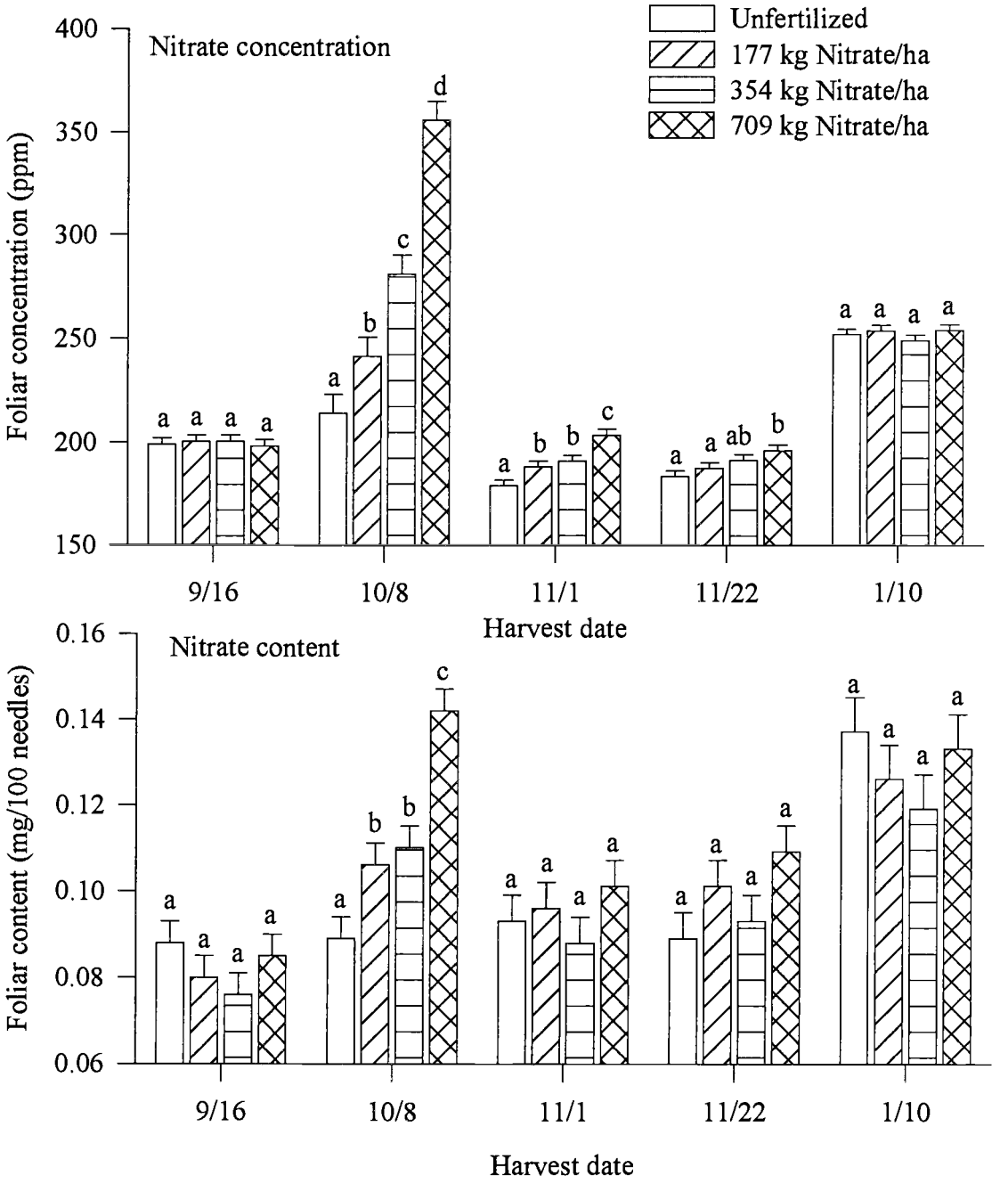


FIGURE 7. Foliar nitrate concentrations and contents over time for seedlings fertilized with NH_4NO_3+K_2SO_4. Means followed by different letters within a date were significant at P=0.05.

There was a significant fertilizer type by fertilizer rate by time interaction on foliar nitrate concentrations and contents (P=0.0003 and 0.0097). Seedlings fertilized with NH_4NO_3 increased most on October 8 (41, 80, 158 ppm, and 0.026, 0.034, 57 mg/ 100 needles, respectively) but also decreased the most on November 1 (53, 90, 152 ppm, and 0.010, 0.022, 0.041 mg/100 needles, Figure 7). The foliar nitrate concentration pattern over time of seedlings not fertilized with NH_4NO_3 was similar to the pattern of foliar TKN, increasing slightly on October 8, decreasing on November 1 and remaining stable to November 22 before increasing 30% by January 10. Foliar nitrate contents only increased slightly over initial September levels by November 22, but increased 28 to 49% to January 10 (Figure 9).

2.4.6 Chloride

Foliar chloride concentrations and contents were higher in seedlings fertilized with KCl and the levels generally increased with increased rates of chloride applied. In the 80, 160, and 320 kg K/ha treatments of KCl, 73, 145, and 291 kg/ha of chloride were applied. Relative chloride levels of seedlings fertilized with KCl showed the greatest uptake of all nutrients (Figure 10).

There was a significant fertilizer type x rate interaction effect on mean foliar chloride concentrations and contents on every sampling date after the initiation of the fertilizer treatments ($P<0.05$). As expected, seedlings fertilized with KCl had higher levels of chloride than those not fertilized or fertilized with K_2SO_4 and the levels observed corresponded to the rates of chloride applied on each date (Figure 10). Initial chloride concentrations and contents for seedlings fertilized with K_2SO_4 ranged from 540 to 580 ppm and 0.214 to 0.237 mg/100 needles. Final values ranged from 440 to 485 ppm and 0.222 and 0.264 mg/100 needles (Figures 8 and 10). At the highest rate of applied KCl , levels in the foliage were as high as 1542 ppm and 0.829 mg/100 needles on November 22 before declining by nearly 50% on January 10. However, significant differences remained among the fertilizer rates (Figure 9).

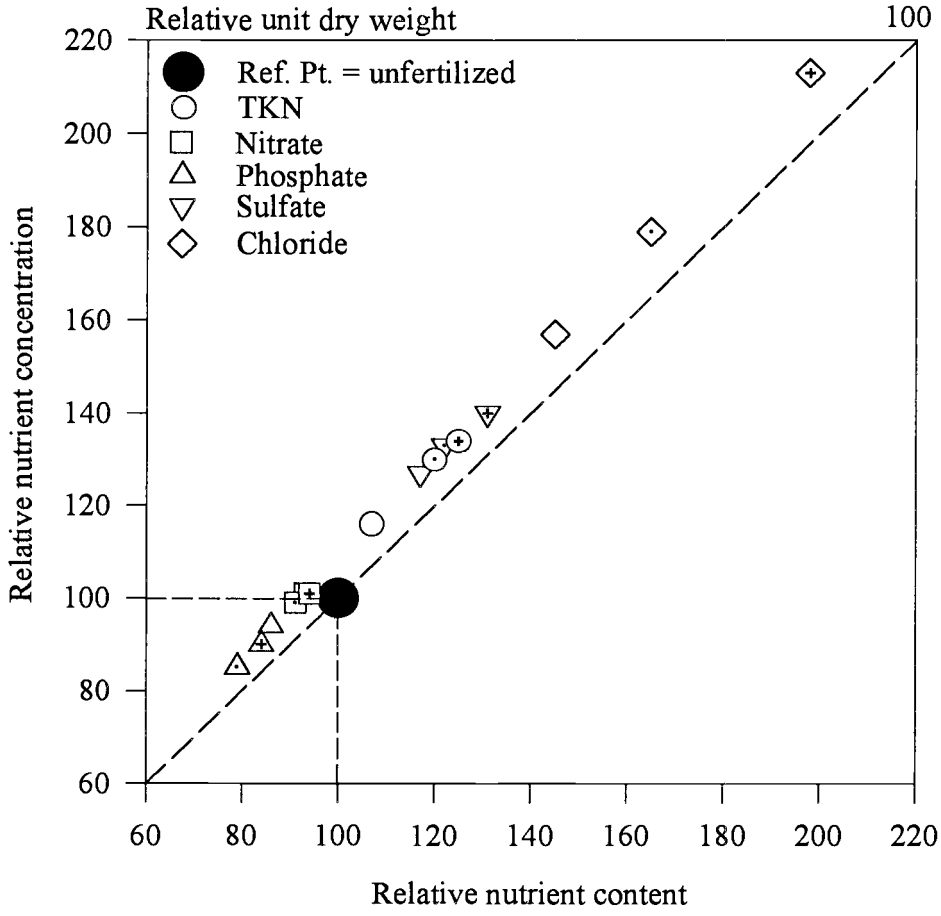


FIGURE 8. Relative TKN and anion shifts among fertilizer rates on January 10. Reference point is the unfertilized nutrient level on January 10. Empty symbols= 80 kg N/ha, dotted=160 kg N/ha, and crossed=320 kg N/ha. Nitrate data include only seedlings fertilized with NH_4NO_3+K_2SO_4. Sulfate and chloride data include only seedlings fertilized with $(NH_4)_2SO_4$+KCl.

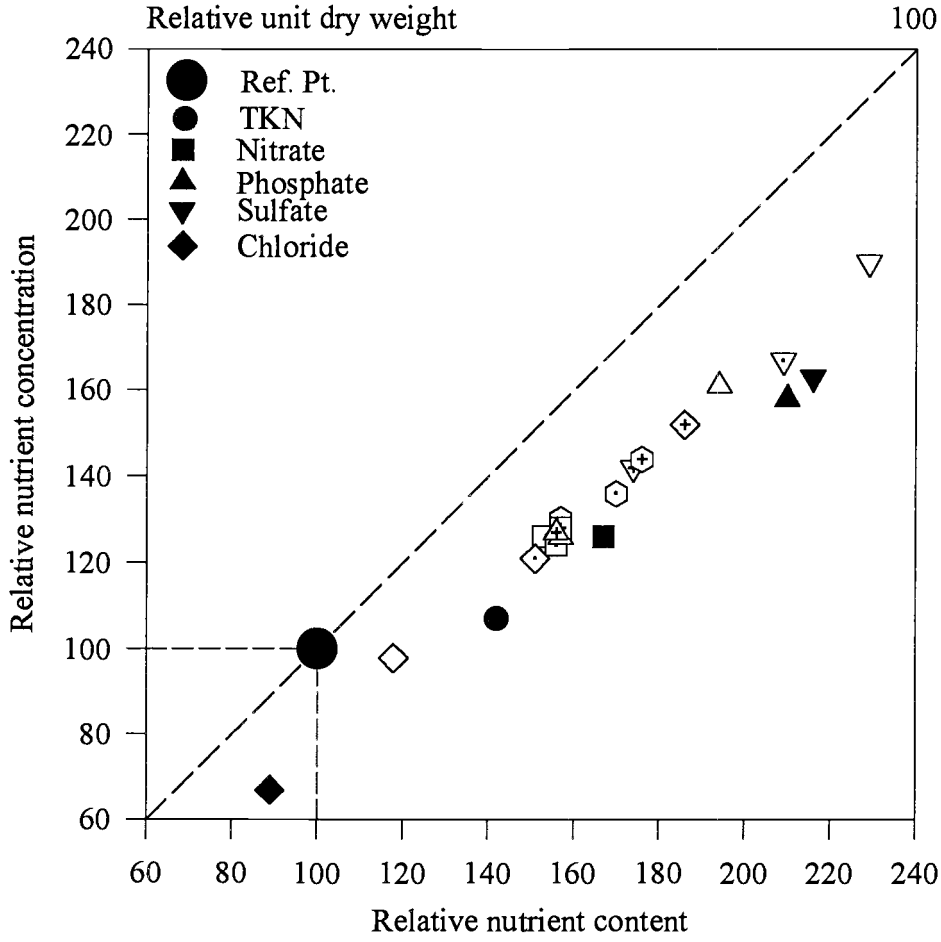


FIGURE 9. Relative TKN and anion shifts between September (reference point) and January by fertilizer rate. The initial September values were set to 100. Filled symbols=unfertilized, empty=80 kg N/ha, dotted=160 kg N/ha, and crossed=320 kg N/ha.

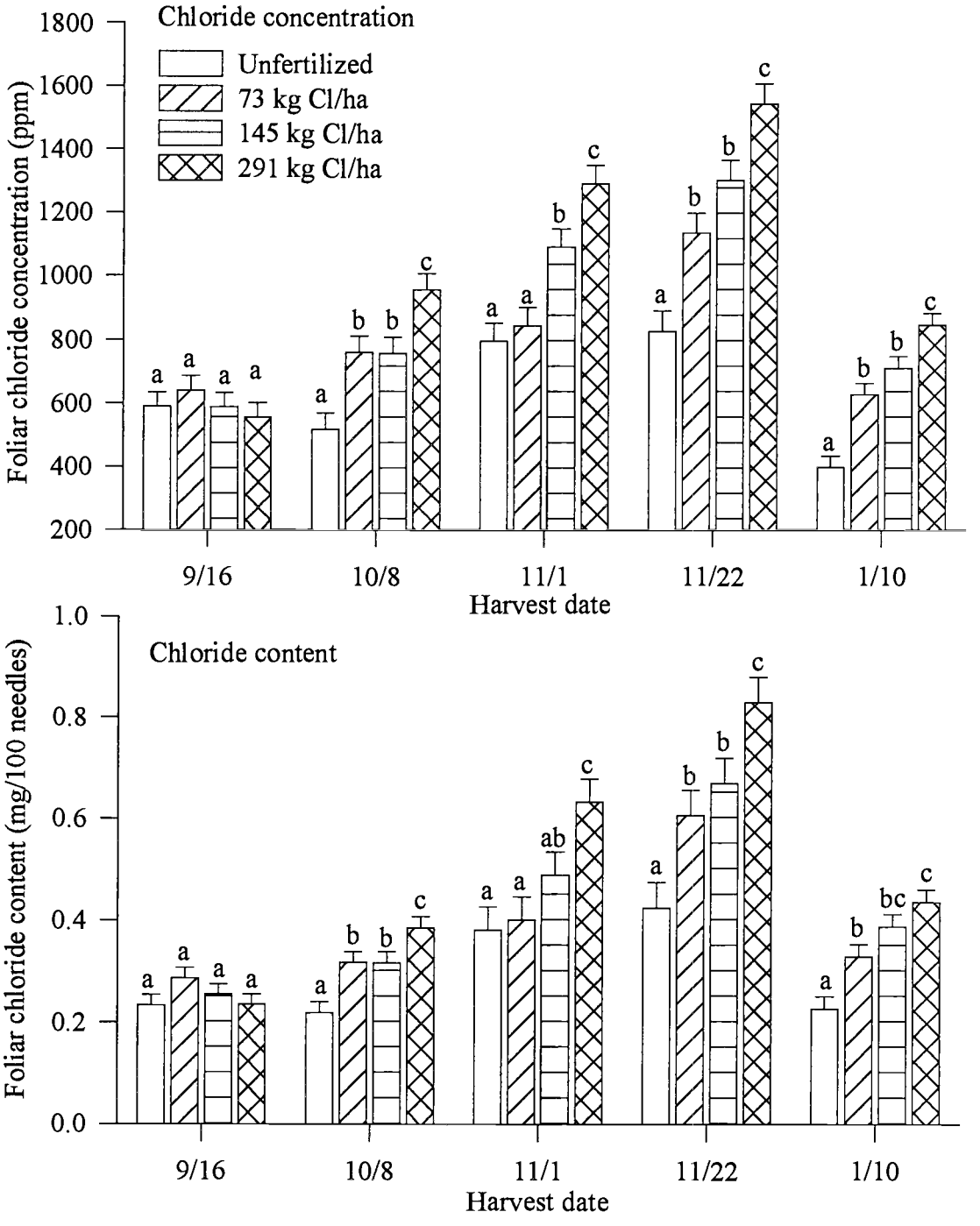


FIGURE 10. Foliar chloride concentration and content over time of seedlings fertilized with $(NH_4)_2SO_4$+KCl. Means followed by different letters within a date were significant at P=0.05.

There was a significant fertilizer type by fertilizer rate by time interaction effect (P=0.0193) on mean foliar chloride concentrations and a fertilizer type by time interaction effect (P=0.0005) on mean foliar chloride contents. The greatest increases in foliar chloride concentrations and contents on October 8, November 1, and November 22 occurred for seedlings fertilized with 320 kg KCl/ha (400, 335, 252 ppm and 0.151, 0.247, 0.197 mg/100 needles, respectively, Figures 8 and 9). These seedlings also decreased the most by January 10 (697 ppm and 0.344 mg/100 needles), but the overall chloride levels remained significantly higher in KCl-fertilized seedlings (625 to 844 ppm and 0.328 to 0.435 mg/100 needles) than in seedlings fertilized with K_2SO_4 (440 to 485 ppm and 0.222 to 0.264 mg/100 needles).

2.4.7 Potassium

Foliar potassium concentrations and contents changed only slightly as a result of applying 80 to 320 kg K/ha and were the least affected of all the nutrients examined in this study.

On November 22, seedlings fertilized with NH_4NO_3+ K_2SO_4 had slightly, but significantly, more foliar K (0.85%) than those fertilized with $(NH_4)_2SO_4$+ KCl (0.81%) (P=0.0143). Seedlings fertilized with K_2SO_4 consistently had slightly higher K concentrations than seedlings fertilized with KCl. There were no significant treatment effects on foliar K contents and no such trends as were exhibited for K concentrations. Although K levels were not significantly different on January 10, a vector diagram depicts the relative differences (Figure 11). Compared to unfertilized seedlings (reference point), seedlings fertilized with 80 kg K/ha showed slightly greater relative response in K concentration and content than seedlings fertilized with 160 and 320 kg K/ha.

There was a significant effect of time on foliar K concentrations (P=0.0086) and a fertilizer type by time interaction effect on foliar K contents (P=0.0390). Contents increased most for seedlings fertilized with NH_4NO_3+ K_2SO_4 to 4.4 mg/100 needles to November 1 and then remained stable. Seedlings fertilized with $(NH_4)_2SO_4$+ KCl

increased more gradually to 4.1 mg/100 needles on November 1 and continued to increase to 4.7 mg/100 needles by January 10.

2.4.8 Sulfate

Over twice as much sulfate was applied as $(NH_4)_2SO_4$ (208, 416, and 832 kg/ha) than as K_2SO_4 (98, 196, and 393 kg/ha), thus seedlings receiving $(NH_4)_2SO_4$ generally had higher sulfate levels. However, this difference was significant on only one date for both sulfate concentrations and contents. On January 10, the sulfate response to fertilization with $(NH_4)_2SO_4$ was similar to, but slightly higher than, the response of TKN (Figure 5).

On October 8, seedlings fertilized with $(NH_4)_2SO_4$+ KCl had significantly higher sulfate concentrations than those fertilized with NH_4NO_3+ K_2SO_4 (1579 vs. 1172 ppm) (P=0.0228). Sulfate contents of seedlings fertilized with $(NH_4)_2SO_4$+ KCl were significantly higher (0.652 mg/100 needles) on this date as well than in NH_4NO_3+ K_2SO_4 fertilized seedlings (0.474 mg/100 needles) (P=0.0180). This trend continued through January 10 for both sulfate concentrations and contents. On January 10, relative foliar sulfate differences among seedlings fertilized with $(NH_4)_2SO_4$ corresponded to the fertilizer rates (Figure 8).

Sulfate concentrations and contents significantly increased over time from approximately 1300 ppm and 0.65 mg/100 needles on September 16 to 2500 ppm and 1.30 mg/100 needles by November 22. Concentrations and contents declined to 2100 ppm and 1.14 mg/100 needles by January 10 (P=0.0001). There were no fertilizer type nor fertilizer rate effects on these changes over time. Figure 9 depicts these changes over time relative to the initial September levels.

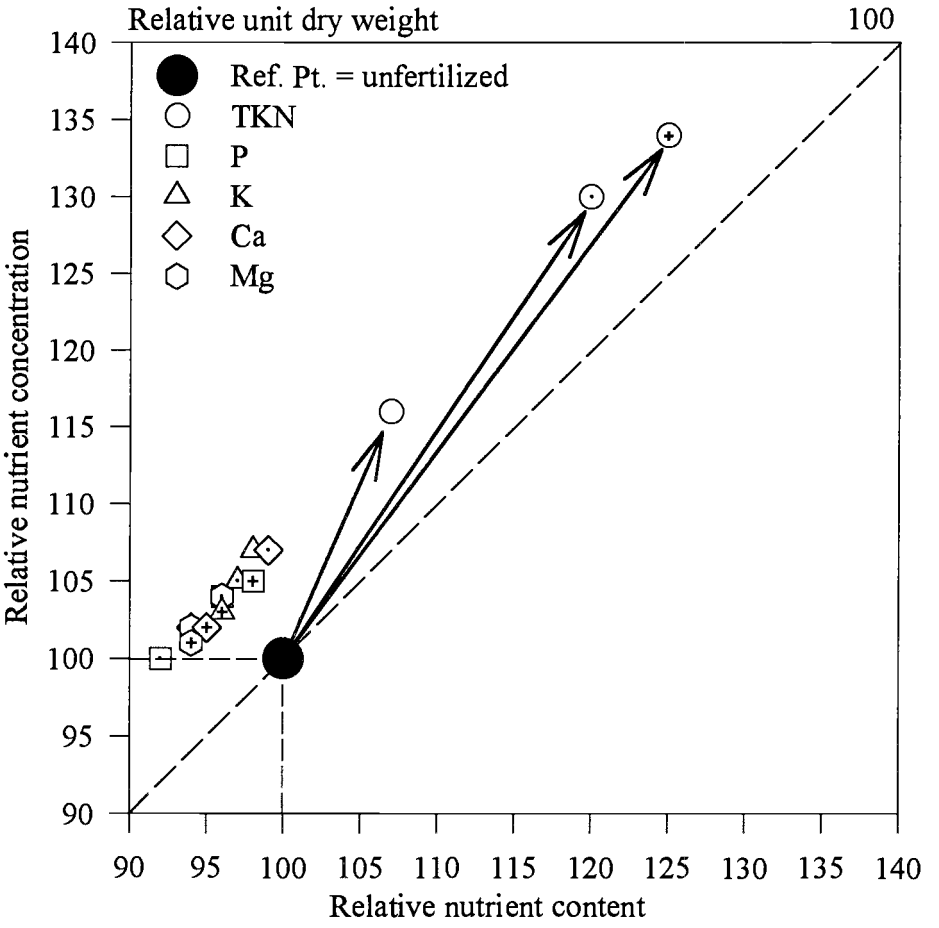


FIGURE 11. Relative macronutrient shifts among fertilizer rates on January 10. Reference point is the unfertilized nutrient level on January 10. Empty symbols=80 kg N/ha, dotted=160 kg N/ha, and crossed=320 kg N/ha. Relative TKN differences among the unfertilized control. 80 kg N/ha, and 160 kg N/ha treatments were significant at P=0.05.

2.4.9 Other Nutrients

Fall applications of nitrogen and potassium did not consistently affect concentrations and contents of the other nutrients measured. There were significant fertilizer type by rate interaction effects on Mn, Fe, Cu, Zn concentrations and phosphate and Fe contents; a fertilizer type effect on P and Ca concentrations and P content; and a fertilizer rate effect on Ca, B, and phosphate concentrations. These differences occurred on only one date for each of the above nutrients and there were no consistent prior or subsequent trends (means separation in appendix tables Va and Vb). Since levels of these nutrients changed over time (as did needle dry weights), mean concentrations and contents of each nutrient averaged over all treatments are presented (Table 3).

TABLE 3. Nutrient concentrations (% and ppm) and contents (mg/100 needles) over time. Note: there were no consistent differences among the treatments for these nutrients. Thus, all treatments were combined to present the trends over time.

Date	DW (mg/100 needles)	% P	P content	% HPO4	HPO4 content	% Ca	Ca content	% Mg	Mg content	Mn ppm	Mn content
16-Sep	420	0.12	0.49	0.15	0.61	0.30	1.27	0.12	0.50	108	0.05
8-Oct	410	0.12	0.48	0.15	0.61	0.33	1.39	0.12	0.49	119	0.05
1-Nov	490	0.15	0.73	0.19	0.93	0.38	1.86	0.13	0.63	170	0.08
22-Nov	520	0.16	0.85	0.23	1.22	0.41	2.13	0.13	0.69	170	0.09
10-Jan	530	0.18	0.94	0.21	1.09	0.40	2.08	0.13	0.67	190	0.10

Date	Fe ppm	Fe content	Cu ppm	Cu content	B ppm	B content	Zn ppm	Zn content
16-Sep	328	0.137	3.62	0.0015	17.00	0.007	25.06	0.011
8-Oct	166	0.069	3.84	0.0016	17.91	0.007	25.00	0.010
1-Nov	386	0.187	5.81	0.0028	19.81	0.010	23.16	0.011
22-Nov	487	0.253	5.63	0.0029	19.78	0.010	23.84	0.012
10-Jan	292	0.152	5.25	0.0027	23.13	0.012	31.75	0.017

On January 10, changes in P, K, Ca, Mg, Fe, Cu, B, and Zn levels of fertilized seedlings relative to unfertilized seedlings were slight (Figures 11 and 12). Only Mn was negatively (though nonsignificantly) affected by the rate of fertilizer applied (Figure 12).

(NOTE: The shift left is because the unfertilized seedlings had a greater (though nonsignificant) needle weight on January 10. Each symbol should really be on about the same relative unit weight axis). Graphical presentation of the relative shifts in nutrient levels as TKN increased between the initial and final harvest clearly show how the increase in nutrient levels over time were largely unaffected by the rates of N and K applied (Figures 13 and 14). On a relative basis, P and Mn increased most over time. Potassium and Mg increased least. Only Fe showed a slightly negative response over time (Figure 14).

2.4.10 Recovery of Applied Nitrogen

The apparent nitrogen recovered in this study is an underestimation of the actual nitrogen recovered since total nitrogen per seedling was not determined (stems and roots were not included). Therefore, comparisons between treatments can only be made on a relative basis. Apparent nitrogen recovery for the 80, 160, and 320 kg N/ha rates were 8.5%, 13.4%, and 8.9%, respectively.

2.4.11 Root Growth Potential and Bud Break Timing

There were no significant treatment effects on new root growth by the end of the root growth potential trial. The mean weight of fresh new root growth at day 32 was 5.1 g per seedling regardless of treatment. Since the terminal bud of only four of the 96 seedlings had broken by the termination of the trial, lateral bud break timing is reported here. Bud break was recorded for the seedlings beginning at day 20 and four more times every two to three days until day 32. Seedlings fertilized with 160 and 320 kg N/ha broke bud after approximately 28 days whereas those fertilized with 80 kg N/ha broke bud after 30 days and unfertilized seedlings broke bud after 31 days ($P=0.0279$) (Figure 15). By the end of the trial, all treatments had six to ten seedlings (out of 12) with bursting lateral buds.

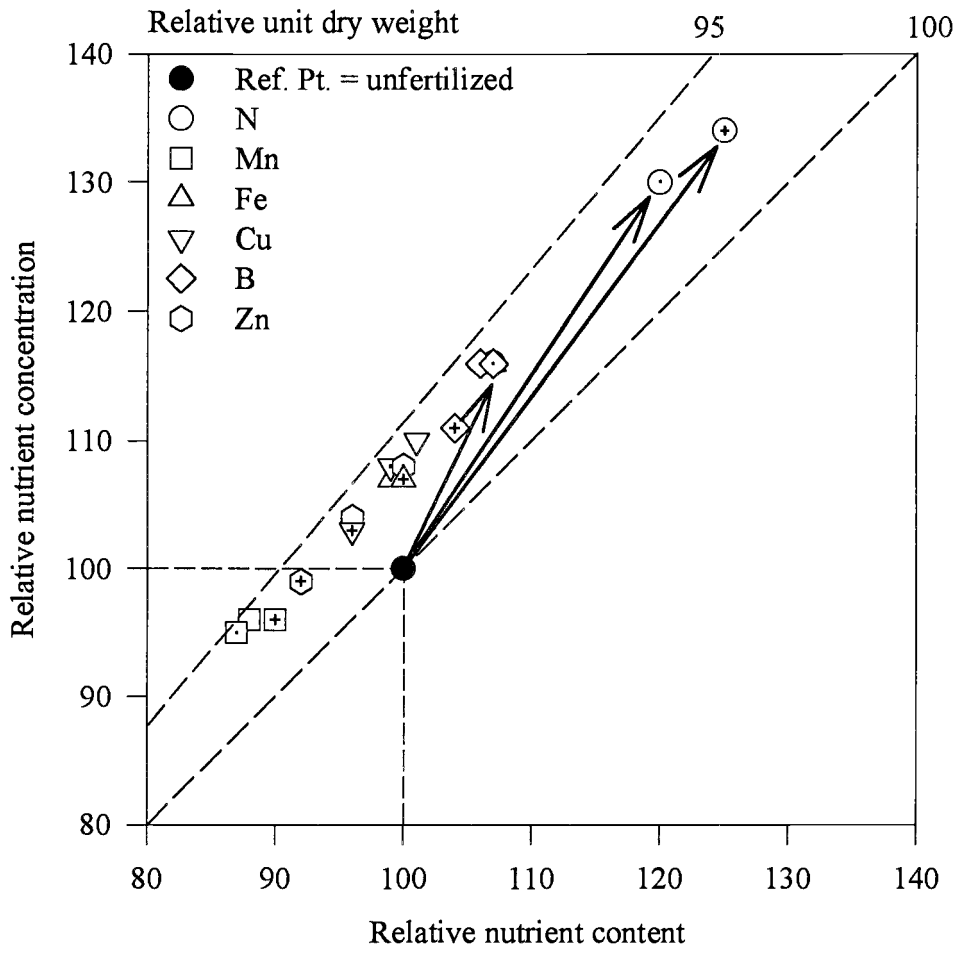


FIGURE 12. Relative micronutrient shifts among fertilizer rates on January 10. Reference point is the unfertilized nutrient level on January 10. Empty symbols=80 kg N/ha, dotted=160 kg N/ha and crossed=320 kg N/ha.

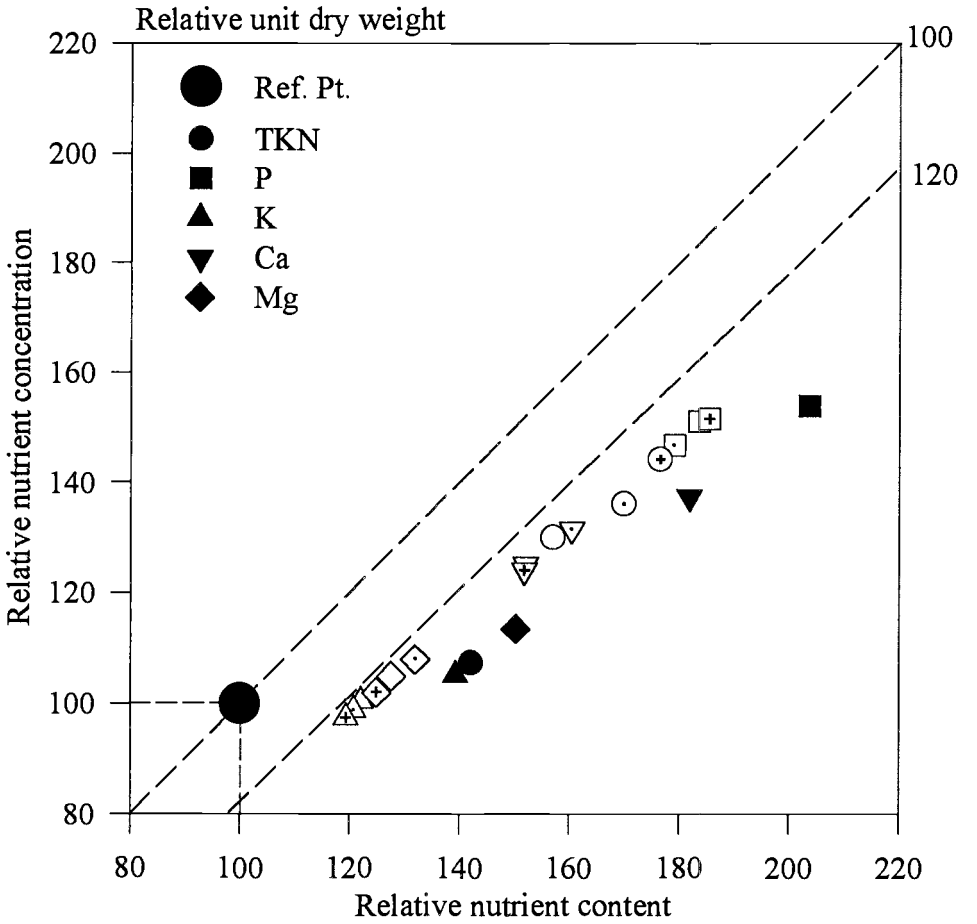


FIGURE 13. Relative macronutrient shifts between September (reference point) and January by fertilizer rate. The reference point is the initial September value of each respective nutrient set to 100. Filled symbols=unfertilized, empty=80 kg N/ha, dotted=160 kg N/ha, and crossed=320 kg N/ha.

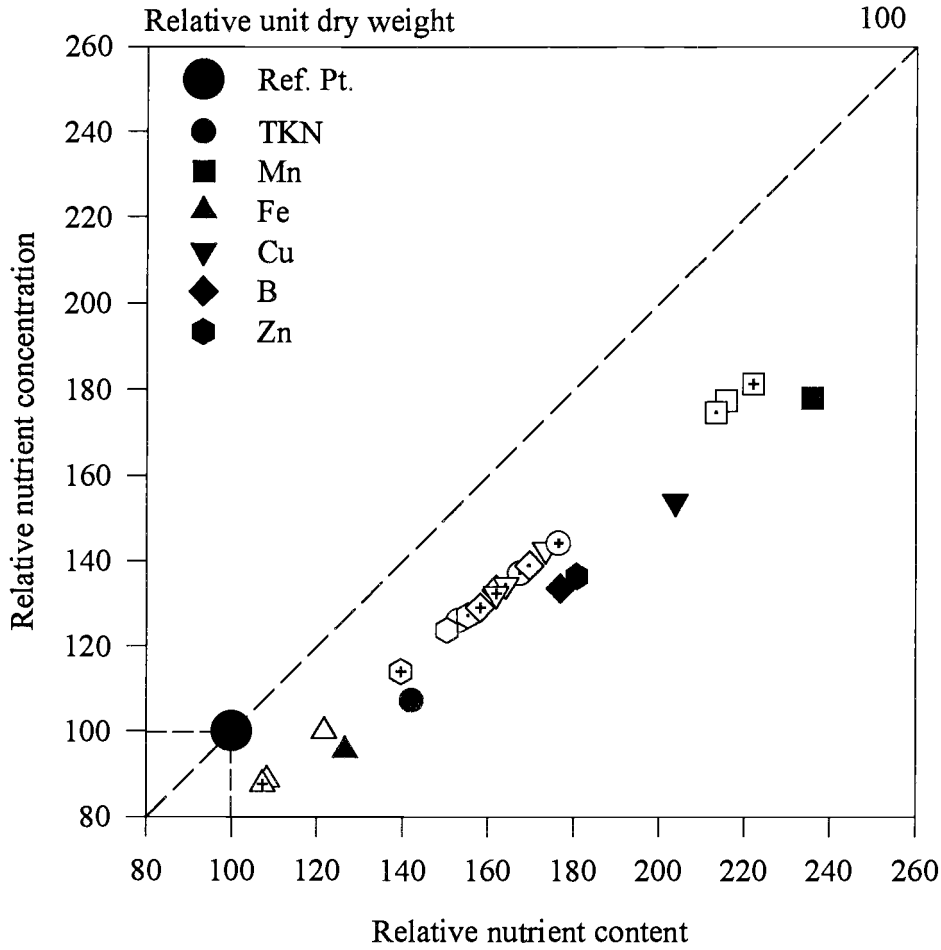


FIGURE 14. Relative micronutrient shifts between September (reference point) and January by fertilizer rate. The reference point is the initial September value of each respective nutrient set to 100. Filled symbols=unfertilized, empty=80 kg N/ha, dotted=160 kg N/ha, and crossed=320 kg N/ha.

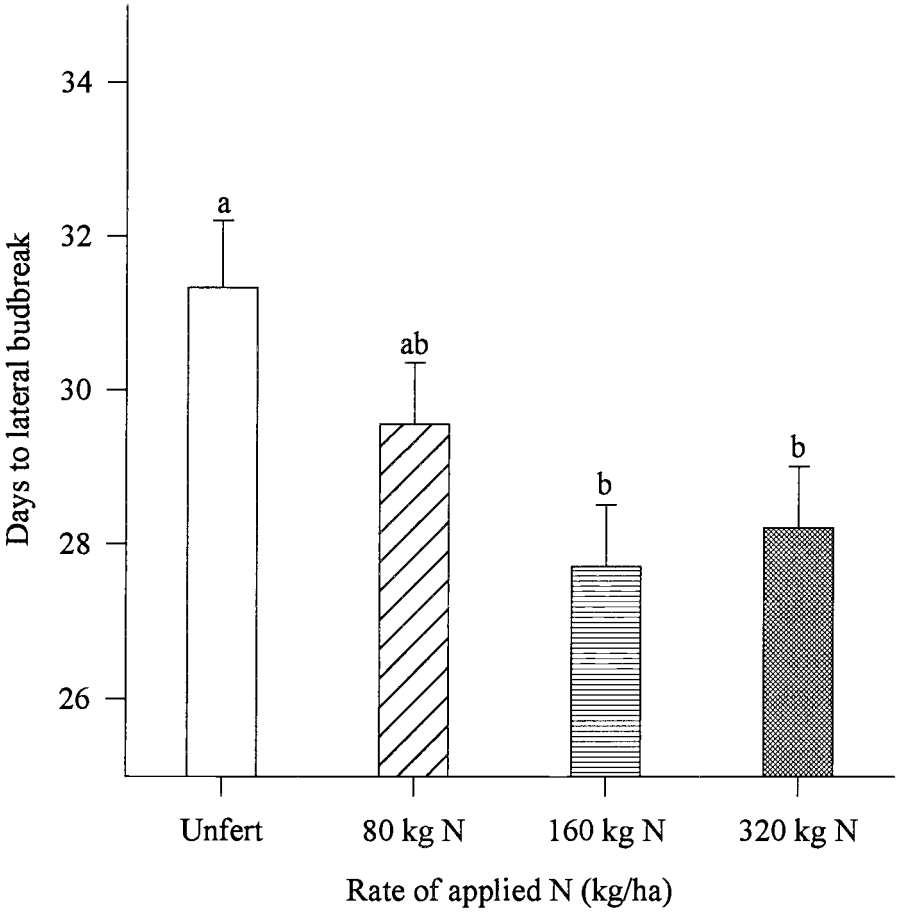


FIGURE 15. Mean number of days to lateral budbreak by fertilizer rate. Means followed by different letters were significant at P=0.05.

2.5 Discussion

2.5.1 General Discussion

A general trends diagram depicts the difference between TKN, chloride, sulfate, and nitrate contents (mg/100 needles) of the intermediate fertilizer rate (160 kg N/ha) and the unfertilized seedlings (Figure 16). Sulfate showed the sharpest increase over time, although it declined by the final harvest date. Clear differences between the two fertilizer rates for both TKN and chloride contents were readily apparent. TKN contents at the 160 kg N/ha rate steadily increased on each successive harvest date, although it appears that the greatest single increase occurred between October 8 and November 1. The TKN contents of unfertilized seedlings increased to November 1, but then leveled off.

Although foliar nitrate contents were significantly differences on October 8, the difference was not real large and nitrate levels did not tend to fluctuate much between September 16 and January 10. Foliar contents of phosphorus, potassium, calcium, and magnesium all increased over time (Figure 17). Again, the sharpest increase for these nutrients occurred between October 8 and November 1. This increase was concurrent with an increase in needle dry weights. It is interesting to note the relatively constant foliar K content.

Although some studies have shown a link between fall fertilization with nitrogen and earlier bud break (Benzian et al. 1974, Thompson 1983, van den Driessche 1985, and Margolis and Waring 1986) this study provides statistical evidence of that link.

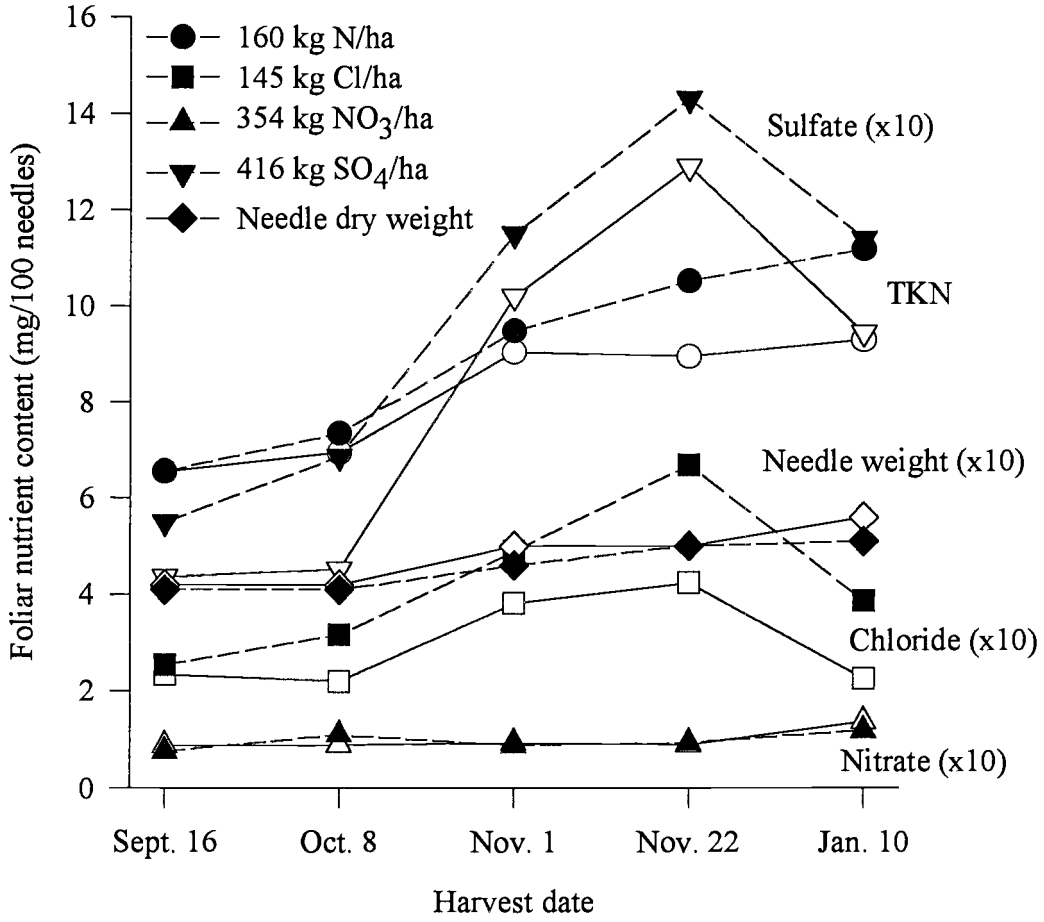


FIGURE 16. Generalized trends diagram of TKN, chloride, nitrate, and sulfate foliar contents and needle dry weights over time. Unfertilized seedlings (empty symbols) are compared to those of the intermediate (160 kg N/ha) rate. The specific amounts of each respective nutrient added is included.

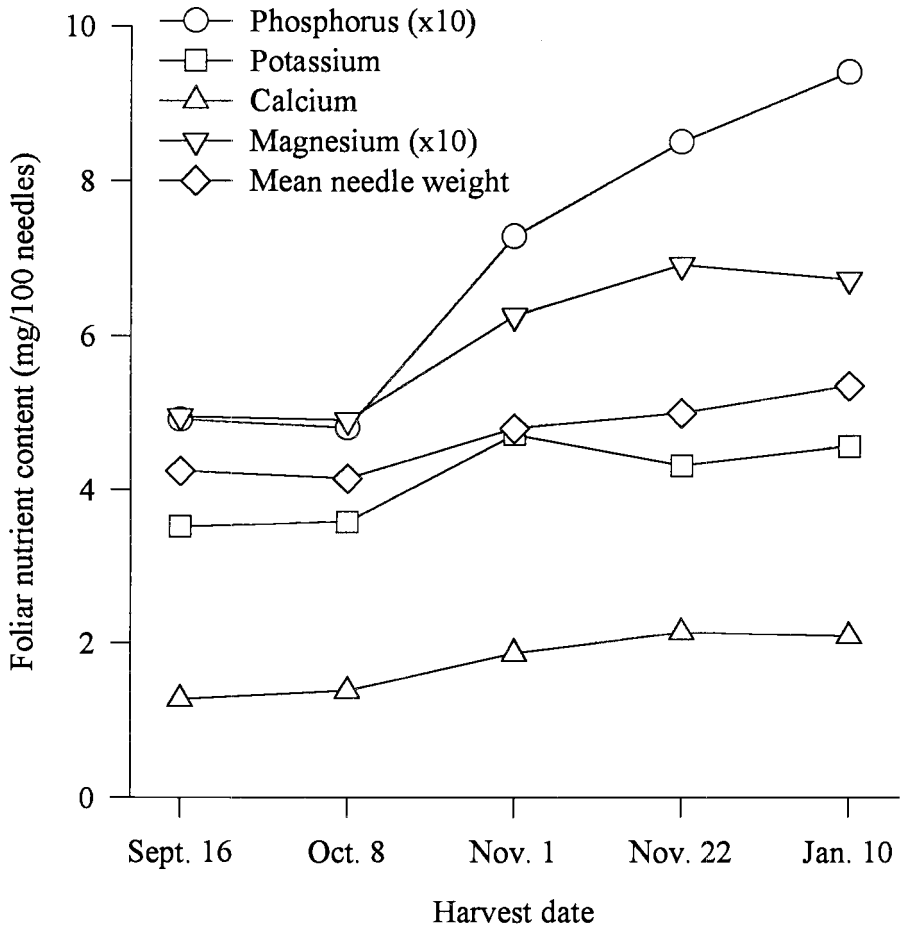


FIGURE 17. General trends in foliar macronutrient contents and mean needle weights over time. Each point is the mean of all treatments.

2.5.2 Foliar TKN

These data suggest that Douglas-fir seedlings continued to take up and translocate nitrogen over the course of the fall and winter and that luxury uptake of nitrogen occurred.

September TKN concentrations were slightly lower than those reported by van den Driessche (1984) for 2+0 Douglas-fir in October and the N and P levels (1.56, 0.12%) were substantially lower than the N and P levels (2.00, 0.38%) reported by Krueger (1967) for a September harvest of 2+0 Douglas-fir at the D.L. Phipps Nursery. But by January, the nitrogen levels of the fertilized seedlings were well above the September concentration and content. By January 10, the 80 kg N/ha treatment led to a significant 16% increase in foliar TKN concentration. The 160 and 320 kg/ha treatments led to a 30 and 34% increase in foliar TKN concentration and a 20 and 26% increase in foliar TKN content over the unfertilized seedlings. This increase suggests luxury uptake of nitrogen occurred. These increases in foliar TKN concentrations are similar to the approximate 37% increase reported by Margolis and Waring (1986) after a February harvest of 2+0 seedlings fall- fertilized with 112 kg N/ha and the approximate 40% increase reported by van den Driessche (1985) after a January harvest of 2+0 Douglas-fir seedlings fall-fertilized with 160 or 320 kg N/ha. The slightly lower response observed in our study may be due to the higher initial TKN levels.

The increases in nitrogen obtained after fall fertilization were in the ranges reported (23-78%) after exponential fertilization or nutrient loading white and black spruce seedlings during the growing season (Miller et al. 1994, Malik and Timmer 1995). These levels, however, are far short of some of the maximum levels reported for 1+0 coastal and interior Douglas-fir (2.55%, 2.79%) (van den Driessche 1984) or that of 2+0 Douglas-fir (Youngberg 1984). Higher levels may be attained if fertilization is started earlier. However, the risk of lammas growth and disruption of the seedling dormancy cycle would be increased. It also appears that the upper rate of applied nitrogen was more than enough, given the lower percentage recovery of applied nitrogen than the 160 kg N/ha rate.

Foliar TKN levels increased over time for all rates, including the unfertilized seedlings, over the initial September levels. Concentrations increased 7, 30, 36, and 44%, and contents increased 42, 56, 71, and 77%, respectively for the 0, 80, 160, and 320 kg N/ha rates (Figures 1 and 3). However, foliar TKN concentrations of unfertilized controls reached a peak in November (16% increase over September levels) before declining 9% by January. TKN contents remained stable at this time. Thus, substantial uptake and/or translocation of nitrogen occurred in the fall, even for the unfertilized seedlings. This slight decline by the final harvest date may signify the end of rest and perhaps a remobilization of nitrogenous substances associated with the fulfillment of the chilling requirement. An examination of mitotic activity and root growth activity during this time would aid in exploring the link between foliar nitrogen levels and these activities during the "dormant" season.

An interpretation of the vector graphs (Figures 3 and 4) is luxury consumption of nitrogen. Luxury consumption occurs when uptake of nutrients is not accompanied by an increase in dry weight. In this instance, nitrogen uptake was accompanied by slight needle dry weight increases. This caused the slight deviation from true luxury consumption interpretation (Timmer and Stone 1978). Large increases in needle weights during the fall were not expected as the seedlings were not actively growing. The small increases over time observed here were not related to the treatments applied, thus nitrogen was not deficient and did not limit growth. Therefore, the TKN increases observed in the fertilized seedlings above those observed in the unfertilized seedlings should be interpreted as luxury consumption.

Nutrient ratios, although significantly different in most instances for the 320 kg N/ha treatment, were generally within the ranges as determined from the concentrations reported by Krueger (1967) (Table 2). At the high rates of fertilizer application, the ratios of P, K, Ca, Mg to TKN were at the low end, and Fe at the high end. Copper and Mn were slightly above below and B was above the ranges as determined from the data of Krueger (1967). Thus, even though TKN concentrations significantly increased from 1.67% to 2.24% by January 10 for the 320 kg N/ha treatment, the nutrient ratios did not become imbalanced and generally fell within the ranges reported by other workers (van

den Driessche 1984). This suggests that the other nutrients were readily available over time and were taken up nearly proportionately to nitrogen and that nitrogen was not a limiting factor to continued nutrient uptake at this time. Any increase above that observed here may have led to the creation of a nutrient imbalance, especially with respect to potassium, manganese, and copper. Indeed, at the high N application, slight K deficiency may have been created. This may become more important when seedling growth resumes as K nutrition becomes more important.

2.5.3 Root TKN

There was a significant difference in root TKN concentrations between unfertilized and fertilized seedlings on October 8 (after 1/3 of the fertilizer had been applied). However, these levels were lower than the initial levels for each rate. This suggests that some translocation of nitrogen to the shoot had occurred between September 19 and October 8. By January 10, root TKN levels in seedlings fertilized with 80, 160, and 320 kg N/ha were 26, 49, and 64%, respectively, greater than levels in unfertilized seedlings. This suggests that luxury uptake was stimulated by the late season fertilizer applications.

It appears substantial nitrogen is stored in the roots and this may have implications for outplanting success. Although root nitrogen levels are not typically analyzed, these results are similar to those reported for the increase in fall-fertilized Douglas-fir fine root nitrogen analyzed in November (64%) and February (50%) (Margolis and Waring 1986) although the actual values in this study were higher (1.41% vs. 0.86%). Also, this study analyzed the total root up to the root collar which suggests dilution. Had less of the stem been included in the analysis, a higher TKN concentration may have resulted. It is interesting to note that the largest differences in root TKN levels among rates occurred over two months (and 1000 mm of precipitation) after the final application of fertilizer. This suggests a relatively long nitrogen residual duration in the rhizosphere. This may be due to low nitrification (which would lead to increased leaching loss of nitrate) because of lower soil temperatures.

Root TKN concentration initially declined on October 8 at the same time foliar TKN concentrations increased relative to initial values. However, the largest increase in foliar

TKN occurred on November 1, but root TKN also increased at this time. Roots were never fully inactive during the fall as evidenced by the presence of white root tips on all harvest dates. Soil temperatures only declined below 5° C for a few days in December and January (Figure III in appendix).

This fall fertilization-stimulated increase in foliar and root TKN concentrations may lead to an increase in N-containing free amino acids which are available for subsequent protein synthesis (and enzymes such as Rubisco). If other required nutrients are available in sufficient proportions and environmental conditions permit, an increase in photosynthetic rates should result. An increase in N-containing free amino acids may or may not lead to an increase in carbohydrates as the metabolic costs of nitrogen assimilation and respiration also increase (Margolis and Waring 1986). Margolis and Waring (1986) found that root carbohydrates declined slightly in fertilized seedlings, but outplanting performance (shoot growth, leaf area, production per unit N) was improved in fall-fertilized seedlings. Survival was apparently unaffected. Further analyses on starch and TNC content changes over time in seedling needles and roots as a result of increased nitrogen concentrations need to be conducted to more fully answer that question.

2.5.4 Nitrate

Although nitrate was not detected in Douglas-fir seedling foliage at any time (Margolis and Waring 1986), in our study, low foliar nitrate concentrations were observed on all sample dates. The significant increase on October 8 followed by the significant decrease by November 1 suggests some nitrate reduction occurs in the foliage since retranslocation of nitrate from the shoots occurs only as amino acids; no nitrate occurs in the phloem (Mengel and Kirkby 1987). Before nitrate can be incorporated into amino acids, it must first undergo reduction to nitrite, followed by further reduction to ammonia.

The immediate effect of the NH_4NO_3 fertilizer on nitrate levels observed on October 8 may have been due to the immediate availability of nitrate at the low soil moisture (nitrate was more available than ammonium because of less ammonium in the soil solution). The buildup of nitrate in the foliage on October 8 was possibly due to the inability of the seedling to reduce large amounts of nitrate until sufficient reducing

enzymes could be induced. Reduction of nitrate did occur as nitrate levels declined by the following harvest. On subsequent harvests, the small differences in nitrate levels between seedlings fertilized with NH_4NO_3 and those fertilized with $(NH_4)_2SO_4$ may be due to the nitrate being rapidly leached from the rhizosphere as a result of large amounts of precipitation before it could be taken up.

In this study, the fraction of nitrate found in the foliage was only a minor fraction relative to TKN (approximately 1 to 2% on a content basis). Little nitrogen is maintained as nitrate; apparently either little nitrate is available in the soil to be taken up by the seedlings or the nitrate that is taken up is rapidly reduced and assimilated into amino acids. Other studies have shown greater growth responses in Douglas-fir seedlings with NH_4-containing fertilizers than NO_3-containing fertilizers (van den Driessche 1971). This may be because of the energy cost associated with reducing nitrate to ammonia prior to amino acid synthesis is greater than the assimilation of ammonium. Also, it may be that more nitrogen is available in the soil as ammonium than as nitrate.

2.5.5 Seedling Needle Weights

Although shoot growth had ceased and there were no treatment effects on mean seedling needle weights over the course of the fall, needle weights continued to increase. The largest increase occurred between October 8 and November 1 (Figure 6) which suggests that needle dry weight accumulation continues late into the fall, after the cessation of active shoot growth, as does caliper and root growth. The needle weights observed in this study are comparable to those reported by Krueger (1967) for October analyzed 2+0 Douglas-fir seedlings from the same nursery (0.414 g/100 needles). It would be interesting to see if needles increase slightly in size at this time, or if the increase is due to an increase in density.

2.5.6 Chloride

Chloride is widely distributed and rapidly recycled and deficiencies are relatively rare. Thus, little attention has been given to chloride levels in tree seedlings. Chloride is

required for the water splitting portion of the Hill reaction (Kelley and Izawa 1978). Chloride is also the counter ion (along with malate) accompanying potassium during its influx into the stomates causing stomatal opening (Mengel and Kirkby 1987, p 574). Concentrations in plants range from 0.2 to 2.0% and 100 ppm is required for biochemical reactions (Agrium 1997).

In mature Douglas-fir trees, concentrations ranged from 73 to 170 ppm (Beaton et al. 1965) and 150 to 1490 ppm (Krueger 1967), but the concentration in seedlings is intermediate with a level of 650 ppm (Krueger 1967). In this study, chloride concentrations ranged from 450 to 820 ppm (0.216 to 0.408 mg/100 needles) for seedlings fertilized with K_2SO_4 and as much as 1550 ppm (0.829 mg/100 needles) for seedlings fertilized with KCl. Although seedlings fertilized with KCl in this study took up nearly twice as much Cl than those not fertilized with a chloride-containing fertilizer, the overall concentration was never higher than 0.16% and there was no evidence of toxicity symptoms such as burning of leaf tips and margins, bronzing, premature yellowing and abscission of leaves (Mengel and Kirkby 1987). This does show how indiscriminately chloride can be taken up.

2.5.7 Potassium

Potassium levels were the least changed by fertilizer treatments than any of the other nutrients examined and K contents increased only slightly (though significantly) over time. Although there was apparently sufficient quantities of K within the seedlings at this time, the levels were not close to some of the maximum levels reported for 1+0 coastal and interior Douglas-fir seedlings (1.32 to 1.44%) (van den Driessche 1984). The increase over time in K contents from 0.43 to 0.62 mg/100 needles suggest either slight uptake from the soil or retranslocation of K from the roots. However, this increase was unrelated to the fertilizer applied. Potassium need is greatest and most uptake generally occurs during the vegetative growth stage (Mengel and Kirkby 1987, p 434). Little growth response is usually detected in Pacific Northwest nurseries because periodic topdressings of K prevent a decline in soil K levels (van den Driessche 1984). The majority of fall fertilization studies, which applied as much as 284 kg K/ha, showed little or no K uptake

in longleaf pine (Hinesley and Maki 1980, van den Driessche 1985, Gleason et al. 1990) except when K was deficient (0.44-0.55%) in Sitka spruce (Benzian et al. 1974). Potassium concentrations even declined 27% in one instance when 2+0 Douglas-fir were fall-fertilized with 210 or 350 kg N/ha as $(NH_4)_2SO_4$ (van den Driessche 1988).

Fertilization with K is apparently not necessary for Douglas-fir seedlings if the soil level is greater than 0.45 meq/100 g soil (van den Driessche 1984). In this study soil K ranged from 0.55 to 0.82 meq/100 g soil and there were no substantial increases in soil K. There was also no evidence of a depression in Ca or Mg concentrations in the soil as a result of supplying large quantities of K to the soil cation exchange sites. In fact, several plots declined slightly in K. The question then is what happened to all that K? Potassium is not held as tightly to the exchange sites as are the divalent cations Ca and Mg (Mengel and Kirkby 1987, p 434) and thus may have been leached from the rhizosphere (or fixed by the soil).

2.5.8 Sulfate

Concentrations of sulfate generally corresponded to the fertilizer type applied but not rates. Seedlings fertilized with $(NH_4)_2SO_4$+KCl received twice the amount of sulfate and generally contained more sulfate than seedlings fertilized with NH_4NO_3+K_2SO_4. There also may have been some competitive interaction between nitrate and sulfate in seedlings fertilized with NH_4NO_3+K_2SO_4 which also may have affected the uptake of sulfate. The levels of sulfate did not decline with increasing nitrogen concentrations and in fact the ratio of sulfate to TKN increased slightly over time which suggests sufficient sulfate was available in the soil. The relative sulfate response to fertilization of seedlings receiving 80, 160, and 320 kg N/ha as $(NH_4)_2SO_4$+KCl (208, 416, and 832 kg SO_4/ha) was similar to the response of TKN (Figure 8). Like N, sulfur is necessary for the synthesis of cysteine, cystine, methionine and hence for the formation of proteins (Coleman 1966). Therefore, overapplication of N can lead to a deficiency of sulfur (noted by the level of sulfate) and a build up of nitrate. The sulfate pool is that sulfur which remains after all needed sulfur has

been utilized during amino acid synthesis. Thus, the sulfate:N ratio is a more sensitive indicator of nitrogen status of trees than sulfur or nitrogen alone (Turner et al. 1977).

The greatest response to nitrogen fertilization (as urea) in Douglas-fir trees occurred for those trees containing more than 80 ppm sulfate (Turner et al. 1977) or 400 ppm sulfate (Turner et al. 1979). The ratios of sulfate to nitrogen was 3 to 9:100 for current foliage and 6 to 16:100 for year old foliage. In our study the ratio ranged from 7 to 11:100 and concentrations were initially at 1000 ppm and continued to increase through the fall before declining to 2000 ppm on January 10. It is interesting to note that the relative seedling response to sulfate between September 16 and January 10 was greater than the response to nitrogen (Figure 9). Although foliar sulfate levels increased more than TKN (relatively), there were no significant differences based on rate of sulfate applied. It appears that sufficient sulfate was available. However, had sulfate not been applied with the nitrogen, declines in foliar sulfate levels may have resulted. It is suggested to apply nitrogen and sulfur in a ratio of 15:1 to avoid possible sulfur deficiency (Knight 1978). In this study the ratio was 2.4:1 and 1.2:1, apparently much more sulfate was applied than was necessary and this was perhaps the reason for a lack of significant differences among sulfate rates.

2.5.9 Other Nutrients

An important finding in this study is the lack of an effect of increased TKN concentrations (16 to 30%) and TKN contents (6 to 28%) on most other essential nutrients. Only N, K, S, and Cl were added yet uptake and/or translocation continued for most other nutrients into the fall and winter. Uptake and/or translocation of these other nutrients were largely unaffected by the amount of N applied (Figures 11 to 14). Only Mn showed evidence of a decline due to fertilizer rate on January 10. These results suggest that adequate nutrition was available at this nursery and that nitrogen was the nutrient most limiting.

2.5.10 Apparent Nitrogen Recovery

The apparent nitrogen recovery in the foliage of these seedlings was rather low (8 to 13%). This number, while not absolute, is sufficient to confirm that the most efficient rate of fertilization was the 160 kg N/ha. Determination of the absolute amount of N recovered by the seedlings would necessitate evaluating the entire seedling. What this value shows is that the point of diminishing returns is probably between 80 and 320 kg N/ha and that future applications need to be adjusted. Also, the number of applications would undoubtably affect the uptake efficiency and need to be evaluated against the cost of each application, both finanacially and in terms of the somewhat hidden costs such as soil compaction. Numerous seedling fertilization studies have been conducted but few include the apparent nitrogen recovered so that benefit/cost analyses can be conducted. One fall fertilization study reported 17 to 31% of nitrogen applied was recovered in whole longleaf pine seedlings from 172.5 kg N/ha applied. Eleven to 17% of applied nitrogen was recovered from the 345 kg N/ha treatment (Hinesley and Maki 1980). Even at this rate of uptake efficiency, it appears that substantial applied nitrogen is lost, perhaps due to immobilization and leaching.

2.5.11 Root Growth Potential and Bud Break Timing

The lack of a significant difference in root growth between the treatments could be due to the ability of all seedlings to perform similarly when placed in an optimum environment (Grossnickle et al. 1991). Differences in root growth due to the fertilization treatments may not occur until after a longer time has elapsed. However, some studies did observe an increase in RGP as a result of increased nutrition. Fall-fertilized Douglas-fir seedlings had greater RGP than conventionally fertilized seedlings (van den Driessche 1988) and the nitrogen concentrations of exponentially loaded white spruce seedlings correlated with increased RGP (Malik and Timmer 1995).

The earlier bud break pattern observed in the fertilized seedlings is similar to the findings of Benzian et al. (1974), Thompson (1983), van den Driessche (1985), and Margolis and Waring (1986). However, lateral buds were observed in this study since no terminal buds had broken by the end of the 32 day trial. Even so, there was a significant

difference in bud break timing among the treatments. Higher seedling nitrogen levels may lead to more efficient retranslocation to the shoot apices in preparation for bud break and shoot elongation. Exponentially loaded white spruce (Malik and Timmer 1995) or black spruce (Timmer and Munson 1991) seedlings did not break bud earlier than conventionally fertilized seedlings nor did fall-fertilized 2+0 ponderosa pine seedlings (Gleason et al. 1990). The biological significance of this slightly earlier budbreak is doubtful. The experimental conditions here were ideal for forcing growth. In natural situations, the environmental changes occurring in the spring are obviously at a much more gradual rate with warming days and cool nights. However, biological significance is doubtful, given the large differences between the experimental and natural conditions.

Seedlings outplanted with elevated nitrogen levels, but with balanced nutrient ratios, may outperform seedlings with lower nitrogen levels. Until intimate root-soil contact is reestablished after outplanting, the seedling can only rely upon the resources existing within it for photosynthetic and metabolic processes. Indeed, planted Douglas-fir seedlings increased in dry matter without any uptake of N and P which suggests the external nutrient supply may be of little immediate importance for initial seedling establishment (van den Driessche 1985). Since current photosynthate appears to be more important for initial growth after outplanting than stored carbohydrates (van den Driessche 1987), the root growth of seedlings able to photosynthesize more efficiently after outplanting should be improved and thus decrease the amount of time needed to establish root-soil contact. This would enhance early seedling survival and growth. Nutrient loaded Douglas-fir seedlings may also take up more nutrients after outplanting than seedlings with adequate nutrient concentrations, much like the preconditioning effect observed in nutrient loaded white and black spruce seedlings (Miller et al. 1994, Malik and Timmer 1995).

Alternatively, if the energy required to assimilate the fall-applied nitrogen into amino acids is greater than the rate of photosynthesis, seedling carbohydrate reserves may be depleted such that seedling vigor is decreased and outplanting performance may be negatively affected (Margolis and Waring 1986).

2.6 Conclusion

The null hypothesis of no treatment effect on foliar nutrient concentrations, contents, and ratios was rejected. The null hypothesis of no treatment effect on root TKN concentrations was also rejected. The null hypotheses of no treatment effect on needle dry weights and root growth potential were not rejected. The null hypotheses of no effect on bud break timing and of no change in nutrient levels between September and January was rejected.

This study was successful in stimulating luxury uptake of nitrogen without drastically altering the balance of other nutrients. Fall fertilization with NH_4NO_3 or $(NH_4)_2SO_4$ is a viable alternative for improving seedling nitrogen nutrition if applied after growth has ceased and buds have set. However, the benefits of this practice cannot be completely described until the link between fall fertilization with nitrogen and outplanting performance is better established.

There appears to be no benefit of applying potassium in the fall if seedlings already contain adequate levels. The similar relative responses in uptake of nitrogen and sulfate emphasize the need to supply sulfur with additional nitrogen. However, in order to better understand the effects of fall increased nitrogen levels, further research on the effects on root, caliper, and needle growth needs examination.

To more fully understand the significance of increased nitrogen as a result of fall fertilization, studies to examine amino acid concentrations, photosynthesis rates, and carbohydrate concentrations during this time need to be conducted. Since linking increased nitrogen with outplanting performance is difficult, perhaps more studies using seedlings potted into a sterilized soil medium in order to examine newly planted (and nutrient loaded) seedling growth and nutrient retranslocation (van den Driessche 1985), or the bioassay approach (Munson and Timmer 1989) could be conducted.

If a link between seedling nutrient status (specifically nitrogen) and outplanting performance can be more clearly elucidated, fall fertilization could become a more common nursery cultural practice. The benefits of a rapidly establishing and vigorous growing seedling is obvious on the reforestation bottom line and on the higher return on

investment at harvest time. Nursery managers would be able to recoup their initial costs of implementing a fall fertilization practice through the sale of higher quality, nutrient-loaded seedlings.

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2.8 Appendix

2.8 Appendix

TABLE I. Bulk fertilizer determination

		Nitrogen fertilizer				Potassium fertilizer				
NH4NO3 + K2SO4	Date	Bulk	NH4	NO3	Total N	Total bulk	K	SO4	S	Cl
80	19-Sep	76.19	17.14	59.05	26.67	59.39	26.67	32.72	10.93	
	11-Oct	76.19	17.14	59.05	26.67	59.39	26.67	32.72	10.93	
	1-Nov	76.19	17.14	59.05	26.67	59.39	26.67	32.72	10.93	
	Total	228.57	51.43	177.14	80.00	178.17	80.00	98.17	32.78	0.00
160	19-Sep	152.38	34.29	118.10	53.33	118.78	53.33	65.45	21.86	
	11-Oct	152.38	34.29	118.10	53.33	118.78	53.33	65.45	21.86	
	1-Nov	152.38	34.29	118.10	53.33	118.78	53.33	65.45	21.86	
	Total	457.14	102.86	354.29	160.00	356.35	160.00	196.35	65.57	0.00
320	19-Sep	304.76	68.57	236.19	106.67	237.56	106.67	130.90	43.71	
	11-Oct	304.76	68.57	236.19	106.67	237.56	106.67	130.90	43.71	
	1-Nov	304.76	68.57	236.19	106.67	237.56	106.67	130.90	43.71	
	Total	914.29	205.71	708.57	320.00	712.69	320.00	392.69	131.14	0.00
(NH4)2SO4 + KCl	Date	Tot. bulk	NH4	NO3	Total N	Total bulk	K	SO4	S	Cl
80	19-Sep	125.79	34.34		26.67	50.89	26.67	69.31	23.14	24.22
	11-Oct	125.79	34.34		26.67	50.89	26.67	69.31	23.14	24.22
	1-Nov	125.79	34.34		26.67	50.89	26.67	69.31	23.14	24.22
	Total	377.36	103.02	0.00	80.00	152.67	80.00	207.92	69.43	72.67
160	19-Sep	251.57	68.68		53.33	101.78	53.33	138.62	46.29	48.45
	11-Oct	251.57	68.68		53.33	101.78	53.33	138.62	46.29	48.45
	1-Nov	251.57	68.68		53.33	101.78	53.33	138.62	46.29	48.45
	Total	754.72	206.04	0.00	160.00	305.34	160.00	415.85	138.87	145.34
320	19-Sep	503.14	137.36		106.67	203.56	106.67	277.23	92.58	96.90
	11-Oct	503.14	137.36		106.67	203.56	106.67	277.23	92.58	96.90
	1-Nov	503.14	137.36		106.67	203.56	106.67	277.23	92.58	96.90
	Total	1509.43	412.08	0.00	320.00	610.69	320.00	831.70	277.74	290.69

Fertilizer	Mol. wt	% NH4	% NO3	% N	% K	% SO4	% S	% Cl	Sol.
NH4NO3	80.00	22.50	77.50	35.00					118.00
(NH4)2SO4	132.10	27.25		21.20		72.75	24.30		71.00
K2SO4	174.30				44.87	55.13	18.42		7.00
KCl	74.60				52.41			47.59	35.00

Element	At.Wt.
Nitrogen	14.00
Hydrogen	1.00
Oxygen	16.00
Sulfur	32.10
Chlorine	35.50
Potassium	39.10

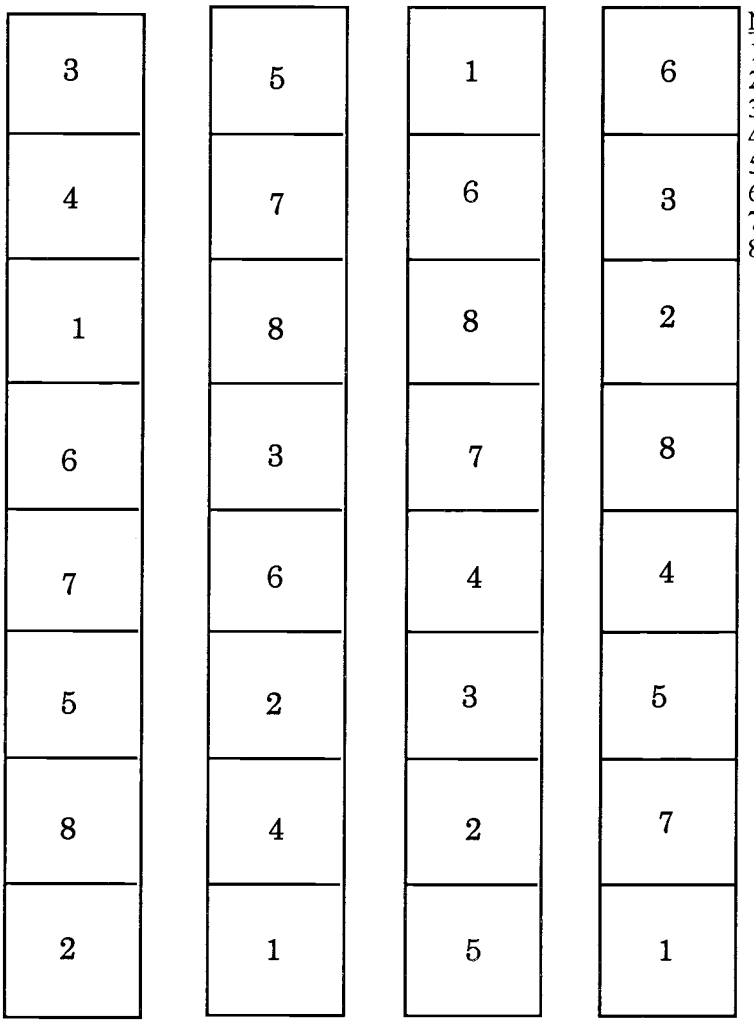
Mole	Mol.wt
NH4	18.00
NO3	62.00
SO4	96.10

TABLE II. Nursery soil data

	NH4NO3 + K2SO4							
	Unfertilized		80 kg/ha		160 kg/ha		320 kg/ha	
	Sept.	Nov.	Sept.	Nov.	Sept.	Nov.	Sept.	Nov.
pH	5.2	5.6	5.0	5.3	5.1	5.2	5.0	5.0
P ppm	42	47	47	50	49	53	50	50
K ppm	222	215	257	238	246	265	222	320
Ca meq	9.5	8.3	8.6	8.1	8.8	7.3	8.3	7.6
Mg meq	2.6	2.4	2.4	2.4	2.5	2.2	2.6	2.2
B ppm	0.3	0.3	0.2	0.3	0.2	0.5	0.2	0.5
Fe ppm	108	128	102	132	106	138	120	132
Mn ppm	11.5	12.2	12.6	11.9	10.5	14.8	11.4	14.4
Cu ppm	1.4	1.4	1.4	1.4	1.3	1.2	1.3	1.5
Zn ppm	0.80	0.90	0.78	0.82	0.74	0.86	0.86	0.86
% C	1.56	1.53	1.62	1.68	1.59	1.59	1.65	1.59
% N	0.12	0.13	0.12	0.13	0.12	0.13	0.13	0.13
% S	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01

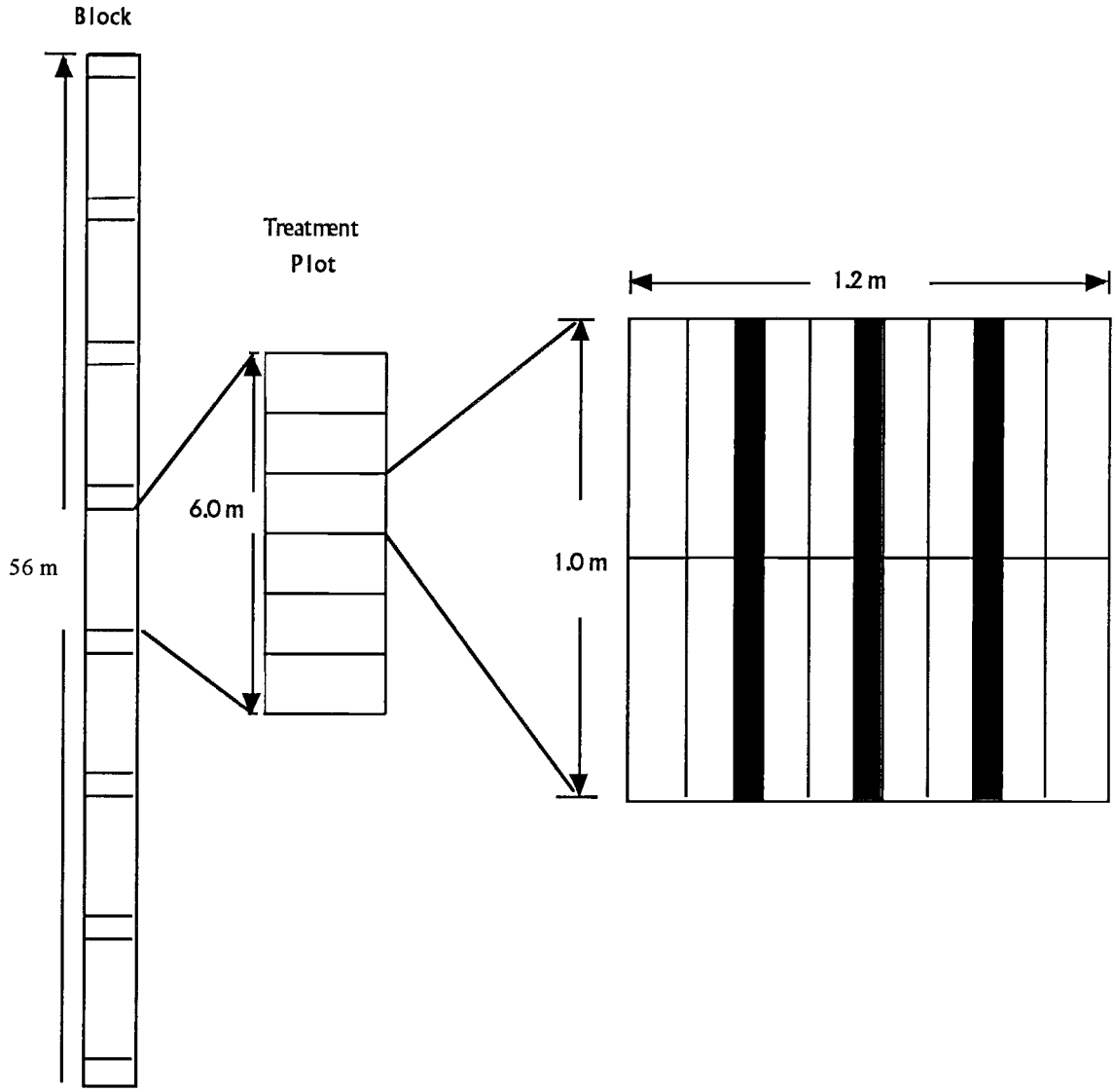
	(NH4)2SO4 + KCl							
	Unfertilized		80 kg/ha		160 kg/ha		320 kg/ha	
	Sept.	Nov.	Sept.	Nov.	Sept.	Nov.	Sept.	Nov.
pH	5.2	5.5	5.0	5.1	5.1	5.0	5.2	5.0
P ppm	66	64	48	51	59	56	52	50
K ppm	293	257	238	230	254	269	261	277
Ca meq	8.2	7.6	8.7	7.6	7.7	6.5	8.1	7.2
Mg meq	2.4	2.3	2.6	2.3	2.3	1.9	2.4	2.1
B ppm	0.2	0.4	0.2	0.3	0.2	0.3	0.3	0.3
Fe ppm	110	148	112	1.38	108	138	114	132
Mn ppm	12.8	16.1	10.5	17.3	8.8	17.3	11.6	14.6
Cu ppm	1.2	1.4	1.4	1.2	1.2	1.2	1.2	1.4
Zn ppm	1.00	1.02	0.84	0.82	0.76	0.82	0.80	0.82
% C	1.67	1.64	1.62	1.53	1.53	1.53	1.67	1.42
% N	0.12	0.13	0.12	0.13	0.12	0.13	0.13	0.12
% S	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.01

Figure I. Treatment plot designations



No.	Fertilizer	Kg N/ ha
1.	NH4NO3+K2SO4	0
2.	NH4NO3+K2SO4	80
3.	NH4NO3+K2SO4	160
4.	NH4NO3+K2SO4	320
5.	(NH4)2SO4+KCl	0
6.	(NH4)2SO4+KCl	80
7.	(NH4)2SO4+KCl	160
8.	(NH4)2SO4+KCl	320

Figure II. Treatment plot layout



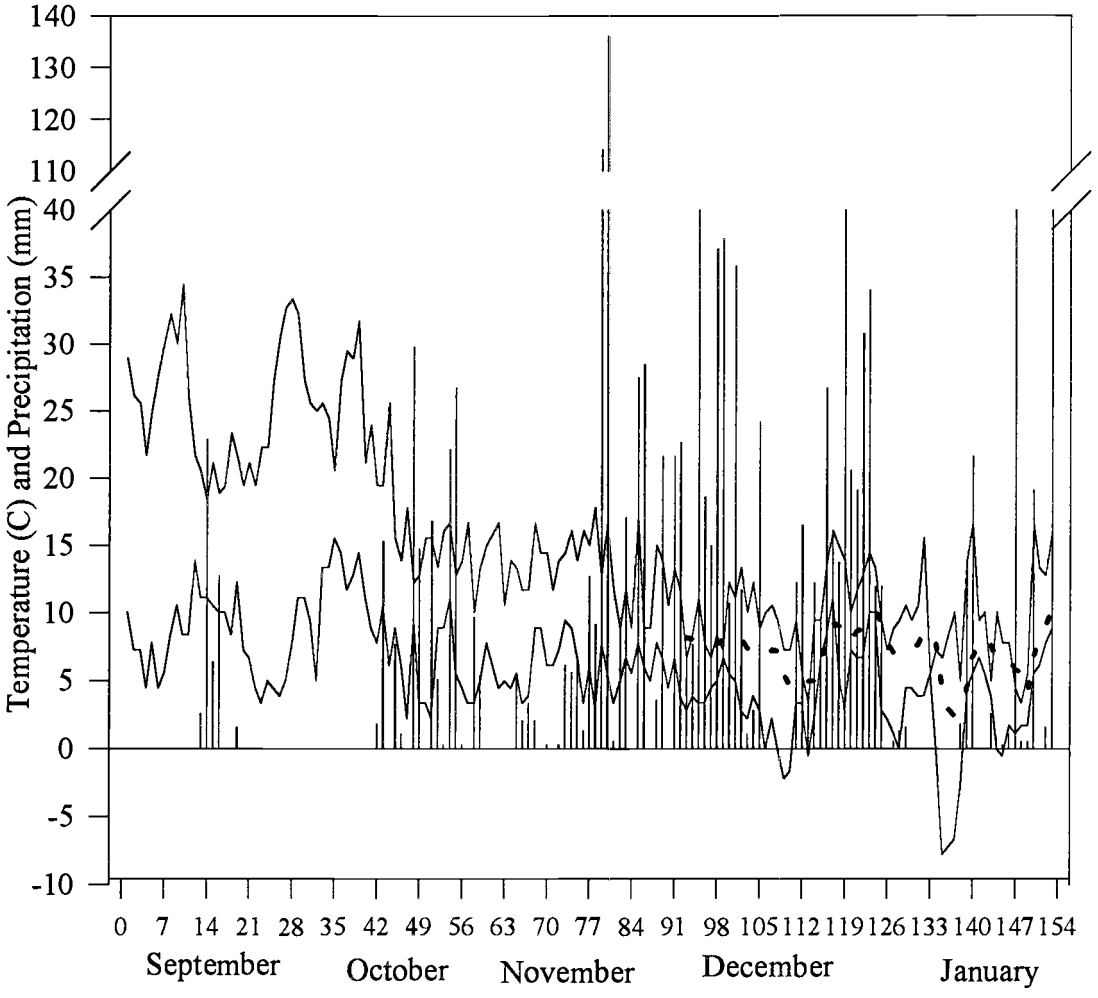


Figure III. D.L. Phipps Nursery precipitation, minimum and maximum air temperature, and soil temperature Sept. 1 to Jan. 31, 1997.

TABLE III a. P-values of nutrient concentrations

Harvest	Source	df	Dry Wt	Rt TKN	TKN	Nitrate	P	Phos	K	Sulfate	Ca	Mg
Sept. 16	Block	3	0.2805	0.0595	0.0001	0.4284	0.0003	0.0001	0.1728	0.0387	0.3412	0.1498
	Fert	1	0.3957	0.3506	0.4093	0.2946	0.1330	0.9137	0.1571	0.2327	0.3573	0.7954
	Rate	3	0.9343	0.7774	0.0707	0.8798	0.4954	0.2511	0.1750	0.6095	0.0730	0.2654
	FxR	3	0.1310	0.2054	0.0994	0.5460	0.6511	0.1248	0.7780	0.6562	0.0659	0.6994
Oct. 8	Block	3	0.2351	0.0008	0.0021	0.6437	0.0031	0.0001	0.2282	0.8063	0.0194	0.1401
	Fert	1	0.7169	0.3346	0.6947	0.0001	0.7815	0.7778	0.2982	0.0228	0.9021	0.8757
	Rate	3	0.4455	0.0065	0.0712	0.0001	0.7765	0.5079	0.2669	0.2419	0.0496	0.8302
	FxR	3	0.5291	0.3927	0.4932	0.0001	0.3040	0.6644	0.8132	0.1378	0.5757	0.4602
Nov. 1	Block	3	0.5511	0.0157	0.0766	0.0237	0.0007	0.0009	0.3386	0.2000	0.0967	0.7203
	Fert	1	0.3747	0.3466	0.6735	0.0014	0.6373	0.4675	0.1742	0.1911	0.1623	0.2281
	Rate	3	0.6025	0.0081	0.0006	0.0025	0.9963	0.6186	0.7808	0.2333	0.5914	0.9422
	FxR	3	0.9637	0.7573	0.8678	0.0038	0.0529	0.0624	0.8058	0.1746	0.6391	0.2333
Nov. 22	Block	3	0.4014	0.5491	0.0012	0.9430	0.0001	0.0003	0.1666	0.0241	0.6128	0.4672
	Fert	1	0.7830	0.4533	0.1384	0.0485	0.8577	0.9490	0.0143	0.1032	0.0122	0.1707
	Rate	3	0.2642	0.0016	0.0003	0.2134	0.1369	0.0130	0.2511	0.3155	0.0081	0.0976
	FxR	3	0.7737	0.9454	0.7838	0.0868	0.0622	0.3749	0.4000	0.3745	0.5799	0.9681
Jan. 10	Block	3	0.7542	0.1673	0.0004	0.2722	0.0039	0.1620	0.3585	0.8857	0.0090	0.0164
	Fert	1	0.2166	0.2098	0.1213	0.0222	0.0237	0.5115	0.5950	0.5706	0.8177	0.5289
	Rate	3	0.4717	0.0001	0.0001	0.9515	0.1441	0.0701	0.2312	0.3585	0.1536	0.6657
	FxR	3	0.6688	0.8372	0.2099	0.1632	0.6835	0.5015	0.5473	0.5317	0.9750	0.1883
	Time		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0086	0.0001	0.0001	0.0001
	TxF		0.3009	0.4972	0.3475	0.0001	0.5606	0.9077	0.1222	0.5473	0.1008	0.5726
	TxR		0.3155	0.0077	0.0016	0.0043	0.5578	0.3189	0.5630	0.7818	0.0043	0.4810
	T x F x R		0.6553	0.4301	0.7275	0.0003	0.4170	0.2785	0.9421	0.7811	0.6310	0.9201

Harvest	Source	df	Mn	Fe	Cu	B	Zn	Cl
Sept. 16	Block	3	0.1242	0.0038	0.4421	0.0291	0.0022	0.9619
	Fert	1	0.2094	0.3287	0.0277	0.3024	0.0743	0.2130
	Rate	3	0.8951	0.3324	0.1308	0.3522	0.0384	0.8511
	FxR	3	0.6752	0.5714	0.5625	0.3539	0.2688	0.6789
Oct. 8	Block	3	0.1883	0.0001	0.0283	0.4903	0.7851	0.1419
	Fert	1	0.7657	0.7033	0.0961	0.7368	0.8044	0.0001
	Rate	3	0.3100	0.0480	0.0518	0.6384	0.0577	0.0103
	FxR	3	0.1920	0.7321	0.0283	0.9995	0.1007	0.0011
Nov. 1	Block	3	0.0476	0.0001	0.6447	0.8205	0.3238	0.3001
	Fert	1	0.7684	0.8757	0.3547	0.1548	0.5058	0.0001
	Rate	3	0.7000	0.3256	0.5644	0.7723	0.8591	0.0004
	FxR	3	0.6166	0.0110	0.5644	0.2501	0.6057	0.0020
Nov. 22	Block	3	0.0024	0.1129	0.4447	0.0012	0.0224	0.2440
	Fert	1	0.6180	0.7198	0.3840	0.1257	0.1263	0.0001
	Rate	3	0.7978	0.4712	0.3486	0.0152	0.4119	0.0003
	FxR	3	0.0272	0.7669	0.3852	0.5098	0.3304	0.0001
Jan. 10	Block	3	0.1231	0.0158	0.2475	0.0477	0.0002	0.1124
	Fert	1	0.6936	0.1699	0.2720	0.5500	1.0000	0.0001
	Rate	3	0.8960	0.3745	0.3869	0.1053	0.0955	0.0001
	FxR	3	0.7660	0.6579	0.1591	0.8527	0.0120	0.0001
	Time		0.0001	0.0001	0.0001	0.0004	0.0481	0.0001
	TxF		0.5650	0.7754	0.0656	0.0345	0.2053	0.0001
	TxR		0.5211	0.0175	0.2877	0.0685	0.0763	0.0489
	T x F x R		0.2190	0.1245	0.6110	0.5455	0.2691	0.0193

TABLE III b. P-values of nutrient contents

Harvest	Source	df	TKN	Nitrate	P	Phos	K	Sulfate	Ca	Mg
Sept. 16	Block	3	0.1851	0.1515	0.0002	0.0001	0.0525	0.0218	0.1156	0.0944
	Fert	1	0.3506	0.2595	0.0344	0.6142	0.0697	0.1653	0.1773	0.3270
	Rate	3	0.9302	0.8949	0.5267	0.2238	0.3333	0.5885	0.2301	0.1868
	FxR	3	0.0668	0.1479	0.1395	0.0047	0.4417	0.5237	0.2839	0.2981
Oct. 8	Block	3	0.0405	0.2130	0.0207	0.0002	0.1263	0.8009	0.0641	0.0740
	Fert	1	0.9720	0.0001	0.6725	0.9333	0.6657	0.0180	0.8641	0.7076
	Rate	3	0.3936	0.0013	0.5347	0.4491	0.4801	0.3380	0.1397	0.7060
	FxR	3	0.3461	0.0002	0.1803	0.4798	0.4955	0.1427	0.4545	0.5922
Nov. 1	Block	3	0.3118	0.3793	0.1174	0.0047	0.5582	0.1262	0.1991	0.5074
	Fert	1	0.5137	0.1020	0.6588	0.8445	0.1651	0.3800	0.1342	0.1732
	Rate	3	0.2338	0.5116	0.6665	0.5350	0.6166	0.2736	0.3478	0.7903
	FxR	3	0.9604	0.9458	0.1225	0.0386	0.9447	0.1486	0.9493	0.6588
Nov. 22	Block	3	0.1192	0.4669	0.0216	0.0065	0.4893	0.0106	0.3961	0.2399
	Fert	1	0.4770	0.8030	0.8606	0.8142	0.4561	0.1166	0.4532	0.5625
	Rate	3	0.0009	0.2326	0.4926	0.2277	0.7179	0.9469	0.2673	0.8509
	FxR	3	0.6929	0.4748	0.5406	0.6657	0.5099	0.3667	0.5699	0.8558
Jan. 10	Block	3	0.0222	0.6482	0.3697	0.6707	0.6944	0.8685	0.3956	0.0591
	Fert	1	0.9779	0.4605	0.0453	0.2216	0.3800	0.3746	0.2305	0.4119
	Rate	3	0.0224	0.5407	0.7049	0.0833	0.9154	0.8461	0.7070	0.6773
	FxR	3	0.2628	0.4878	0.7412	0.6337	0.7031	0.7315	0.6542	0.9642
	Time		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	TxF		0.6560	0.0002	0.2688	0.8721	0.0390	0.8540	0.0062	0.0388
	TxR		0.0039	0.2112	0.6605	0.2795	0.2783	0.4549	0.0061	0.5894
	T x F x R		0.1842	0.0097	0.2904	0.1031	0.8324	0.7403	0.9215	0.6879

Harvest	Source	df	Mn	Fe	Cu	B	Zn	Cl
Sept. 16	Block	3	0.2136	0.0197	0.7344	0.0291	0.1282	0.6422
	Fert	1	0.2149	0.1661	0.0153	0.1248	0.0377	0.1020
	Rate	3	0.9493	0.3354	0.2573	0.3080	0.0904	0.8723
	FxR	3	0.6256	0.5661	0.4301	0.0752	0.4334	0.2320
Oct. 8	Block	3	0.2147	0.0001	0.0166	0.4677	0.8757	0.0876
	Fert	1	0.8706	0.7782	0.2172	0.6422	0.6671	0.0001
	Rate	3	0.5680	0.1588	0.1527	0.9370	0.2505	0.0384
	FxR	3	0.2353	0.9381	0.0598	0.8784	0.1167	0.0029
Nov. 1	Block	3	0.0603	0.0008	0.7921	0.3865	0.8860	0.3532
	Fert	1	0.4497	0.7464	0.7604	0.0899	0.1670	0.0001
	Rate	3	0.4016	0.2310	0.3260	0.5452	0.3555	0.0314
	FxR	3	0.4264	0.0369	0.5703	0.2675	0.3774	0.0435
Nov. 22	Block	3	0.0142	0.1130	0.4364	0.0002	0.0813	0.4500
	Fert	1	0.9576	0.6908	0.4379	0.0732	0.1929	0.0001
	Rate	3	0.6792	0.1751	0.6732	0.1332	0.9288	0.0041
	FxR	3	0.2823	0.9552	0.4169	0.8356	0.6787	0.0073
Jan. 10	Block	3	0.5662	0.0392	0.2109	0.0581	0.0077	0.1193
	Fert	1	0.6354	0.8053	0.7237	0.5129	0.2266	0.0001
	Rate	3	0.4921	0.8031	0.8330	0.8081	0.5996	0.0106
	FxR	3	0.7160	0.3478	0.2484	0.6601	0.3715	0.0006
	Time		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	TxF		0.4048	0.5944	0.0879	0.1419	0.0812	0.0005
	TxR		0.5102	0.0436	0.2864	0.6449	0.1774	0.0910
	T x F x R		0.4552	0.0832	0.1114	0.5972	0.1197	0.0955

TABLE III c. P-values of nutrient ratios

Harvest	Effects	df	Nitrate	P	Phos	K	Sulfate	Ca	Mg
Sept. 16	Block	3	0.0001	0.0001	0.0001	0.0013	0.0121	0.1878	0.0080
	Fert	1	0.8372	0.3935	0.6742	0.4815	0.3004	0.8038	0.7709
	Rate	3	0.0914	0.8498	0.4871	0.3397	0.6699	0.0964	0.2460
	FxR	3	0.0840	0.7384	0.2052	0.5359	0.5892	0.1420	0.7197
Oct. 8	Block	3	0.0545	0.0001	0.0001	0.0621	0.2809	0.3101	0.4775
	Fert	1	0.0001	0.5225	0.9509	0.6249	0.0170	0.7335	0.5996
	Rate	3	0.0004	0.7277	0.0644	0.6574	0.4043	0.4837	0.5879
	FxR	3	0.0002	0.5909	0.8388	0.9494	0.0568	0.6659	0.2818
Nov. 1	Block	3	0.0048	0.0001	0.0003	0.1522	0.0967	0.2445	0.5203
	Fert	1	0.0954	0.9808	0.6646	0.2912	0.4684	0.2401	0.2755
	Rate	3	0.0144	0.0140	0.0470	0.0753	0.1201	0.0064	0.0283
	FxR	3	0.7609	0.0460	0.0622	0.8145	0.2417	0.9830	0.6102
Nov. 22	Block	3	0.0196	0.0001	0.0001	0.0036	0.0018	0.0134	0.0151
	Fert	1	0.6565	0.5140	0.7229	0.7085	0.0641	0.7430	0.8603
	Rate	3	0.0074	0.0004	0.0008	0.0018	0.0376	0.0006	0.0007
	FxR	3	0.6867	0.4006	0.5737	0.6659	0.4786	0.9703	0.8416
Jan. 10	Block	3	0.0027	0.0007	0.0073	0.0476	0.1130	0.0201	0.0044
	Fert	1	0.2544	0.0334	0.1554	0.2312	0.2312	0.1083	0.1513
	Rate	3	0.0001	0.0029	0.0008	0.0030	0.7520	0.0008	0.0001
	FxR	3	0.4817	0.2584	0.3433	0.2781	0.2958	0.3520	0.0335

Harvest	Effects	df	Mn	Fe	Cu	B	Zn	Cl
Sept. 16	Block	3	0.0075	0.0055	0.0589	0.0027	0.0010	0.1516
	Fert	1	0.3810	0.5220	0.0562	0.5719	0.2590	0.3271
	Rate	3	0.8298	0.3541	0.2014	0.5295	0.1053	0.6354
	FxR	3	0.6281	0.5049	0.5013	0.6639	0.3473	0.5381
Oct. 8	Block	3	0.0124	0.0001	0.7703	0.0165	0.0974	0.5402
	Fert	1	0.9857	0.9962	0.2650	0.4936	0.5653	0.0001
	Rate	3	0.8848	0.1771	0.5859	0.7914	0.6259	0.1136
	FxR	3	0.1970	0.6695	0.0600	0.7946	0.1897	0.0008
Nov. 1	Block	3	0.0066	0.0001	0.8950	0.3266	0.3472	0.3311
	Fert	1	0.6621	0.8401	0.4993	0.2124	0.4215	0.0001
	Rate	3	0.0144	0.0986	0.2976	0.0213	0.1642	0.0494
	FxR	3	0.6933	0.0382	0.6116	0.3649	0.6359	0.0063
Nov. 22	Block	3	0.0002	0.0445	0.2955	0.0001	0.0040	0.0101
	Fert	1	0.8551	0.5279	0.4431	0.0302	0.1320	0.0001
	Rate	3	0.0096	0.1286	0.1430	0.0057	0.0731	0.1476
	FxR	3	0.1091	0.6956	0.3396	0.3988	0.6342	0.0004
Jan. 10	Block	3	0.0035	0.0014	0.0026	0.0535	0.0002	0.3979
	Fert	1	0.4133	0.9346	0.5637	0.5352	0.1389	0.0001
	Rate	3	0.0118	0.0372	0.0030	0.1084	0.0017	0.8106
	FxR	3	0.3011	0.4611	0.0574	0.7045	0.0182	0.0011

TABLE IV. Mean squares of nutrient concentrations

Hv	Source	df	Dry Wt	Rt TKN	TKN	Nitrate	P	Phos	K	Sulfate
Sept. 16	Block	3	0.00327	0.03689	0.08349	36.93	0.00213	1242392	0.01228	1091654
	Fert	1	0.00180	0.01163	0.00383	44.29	0.00053	929	0.01445	492917
	Rate	3	0.00034	0.00469	0.01465	8.52	0.00018	113602	0.01219	202575
	FxR	3	0.00525	0.02122	0.01280	27.95	0.00012	165735	0.00246	178247
	MSE	21	0.00240	0.01276	0.00540	38.33	0.00022	77232	0.00671	326435
Oct. 8	Block	3	0.00227	0.02731	0.11411	191.36	0.00101	814079	0.00830	79076
	Fert	1	0.00020	0.00320	0.02628	34339	0.00001	4345	0.00605	1461756
	Rate	3	0.00137	0.01771	0.04494	7685.57	0.00006	42584	0.00751	365022
	FxR	3	0.00113	0.00343	0.01375	7535.40	0.00020	28402	0.00168	496398
	MSE	21	0.00148	0.00328	0.01660	338.28	0.00016	53253	0.00532	242286
Nov. 1	Block	3	0.00375	0.08678	0.06170	128.21	0.00185	1312283	0.00631	363216
	Fert	1	0.00428	0.01853	0.00428	448.85	0.00005	87887	0.01051	392669
	Rate	3	0.00329	0.10249	0.20209	219.98	0.00000	97218	0.00193	331527
	FxR	3	0.00478	0.00791	0.00561	200.69	0.00066	456053	0.00174	391342
	MSE	21	0.00520	0.01999	0.02343	33.06	0.00022	160486	0.00531	215144
Nov. 22	Block	3	0.00334	0.02008	0.12004	3.88	0.00125	957047	0.00285	714573
	Fert	1	0.00025	0.01620	0.03713	134.13	0.00000	404	0.01088	537334
	Rate	3	0.00463	0.20182	0.15811	49.69	0.00020	440365	0.00225	232225
	FxR	3	0.00121	0.00342	0.00560	76.60	0.00027	105316	0.00157	202097
	MSE	21	0.00325	0.02775	0.01564	30.55	0.00010	96587	0.00153	185150
Jan. 10	Block	3	0.00164	0.02450	0.27217	44.38	0.00054	112608	0.00351	43684
	Fert	1	0.00661	0.02205	0.07703	193.96	0.00053	26553	0.00090	67834
	Rate	3	0.00355	0.45844	0.53426	3.60	0.00018	162164	0.00480	231480
	FxR	3	0.00215	0.00373	0.04851	59.96	0.00005	48347	0.00225	154305
	MSE	21	0.00408	0.01317	0.02955	31.82	0.00009	59542	0.00310	204321

Hv	Source	df	Ca	Mg	Mn	Fe	Cu	B	Zn	Cl
Sept. 16	Block	3	0.00094	0.00036	636.21	39353	0.33	33.08	54.21	787
	Fert	1	0.00070	1.2E-05	496.13	6469	2.00	10.13	28.13	13653
	Rate	3	0.00213	0.00026	59.21	7795	0.75	10.42	26.71	2178
	FxR	3	0.00221	8.7E-05	153.38	4429	0.25	10.38	11.21	4232
	MSE	21	0.00079	0.00018	295.88	6470	0.36	9.06	7.97	8277
Oct. 8	Block	3	0.00331	0.00025	776.08	13032	0.95	5.61	2.83	21345
	Fert	1	0.00001	3E-06	40.50	128	0.78	0.78	0.50	376683
	Rate	3	0.00248	3.6E-05	564.83	2674	0.78	3.86	23.25	51158
	FxR	3	0.00055	0.00011	767.67	371	0.95	0.03	18.75	82737
	MSE	21	0.00081	0.00013	444.37	858	0.26	6.73	7.95	10568
Nov. 1	Block	3	0.00206	5.8E-05	2028.62	50671	0.71	1.13	18.86	17469
	Fert	1	0.00180	0.0002	57.78	85	1.13	8.00	7.03	1077639
	Rate	3	0.00056	1.7E-05	395.37	4125	0.88	1.38	3.86	124198
	FxR	3	0.00049	0.0002	311.37	16050	0.88	5.42	9.61	93695
	MSE	21	0.00086	0.00013	649.31	3370	1.26	3.67	15.34	13420
Nov. 22	Block	3	0.00019	8.7E-05	1882.08	16808	3.67	34.03	59.53	23798
	Fert	1	0.00228	0.0002	72.00	3990	3.13	11.28	38.28	1383625
	Rate	3	0.00155	0.00024	95.08	6531	4.58	19.45	15.11	154418
	FxR	3	0.00020	8E-06	1047.25	2861	4.21	3.53	18.28	214001
	MSE	21	0.00030	9.4E-05	281.06	7489	3.95	4.44	15.10	15884
Jan. 10	Block	3	0.00258	0.0003	1786.20	10575	0.58	26.42	59.42	12303
	Fert	1	0.00003	2.8E-05	132.03	4925	0.50	3.13	0.00	257789
	Rate	3	0.00100	3.6E-05	164.53	2662	0.42	19.58	13.92	60247
	FxR	3	0.00004	0.00012	317.20	1325	0.75	2.21	26.92	83419
	MSE	21	0.00052	6.9E-05	827.29	2439	0.39	8.46	5.77	5470

TABLE V a. Means separation of nutrient concentrations

Nutrient	Foliar TKN %					Root TKN %					Ca %				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	1.56	1.65	1.80 a	1.81 a	1.67 a	0.97	0.71a	0.76a	0.84a	0.86a	0.28	0.32 a	0.39	0.41 b	0.39 a
80 kg/ha	1.49	1.78	1.99 b	1.99 b	1.94 b	0.93	0.79b	0.91ab	1.10b	1.08b	0.31	0.35 b	0.39	0.39 a	0.39 ab
160 kg/ha	1.60	1.81	2.09 bc	2.11 bc	2.17 c	0.95	0.78b	0.99b	1.15b	1.28c	0.30	0.35 b	0.37	0.43 b	0.41 b
320 kg/ha	1.56	1.78	2.17 c	2.11 bc	2.24 c	0.92	0.82b	1.01b	1.19b	1.41d	0.32	0.32 ab	0.39	0.41 b	0.39 ab
Std. Error	0.0260	0.0456	0.0541	0.0442	0.0608	0.0399	0.0203	0.0500	0.0589	0.0406	0.0100	0.0100	0.0104	0.0062	0.0080

Nutrient	HPO4 ppm					Dry Weight per 100 Needles					Root Dry Weights				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	1421	1566	2064	2598 c	2249	0.42	0.42	0.50	0.50	0.56	3.33	4.25	5.75	6.50	
80 kg/ha	1310	1458	1910	2463 bc	2106	0.42	0.43	0.49	0.53	0.51	3.15	4.97	5.77	5.51	
160 kg/ha	1523	1389	1985	2253 ab	1914	0.41	0.41	0.46	0.50	0.51	3.31	5.16	5.97	5.90	
320 kg/ha	1581	1460	1806	2065 a	2015	0.42	0.40	0.50	0.55	0.52	3.28	4.66	5.83	5.88	
Std. Error	98.2549	81.5883	141.6361	109.87877	86.27133	0.0173	0.0136	0.0255	0.0217	0.0226	0.2189	0.3233	0.3253	0.3379	

Nutrient	B ppm				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	15.63	17.13	20.25	19.75ab	20.88
80 kg/ha	16.50	17.63	19.88	18.13 a	24.13
160 kg/ha	17.88	18.13	19.88	21.88 b	24.25
320 kg/ha	18.00	18.75	19.25	19.38 a	23.25
Std. Error	1.064	0.917	0.678	0.745	1.029

Nutrient	P %					K %					Ca %				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	0.114	0.116	0.149	0.164	0.175 a	0.82	0.878125	0.898125	0.850a	0.875625	0.30	0.33	0.39	0.42 a	0.40
Fert 2	0.122	0.117	0.152	0.163	0.183 b	0.8625	0.850625	0.861875	0.812 b	0.865	0.31	0.33	0.38	0.40 b	0.40
Std. Error	0.0037	0.0031	0.0037	0.0024	0.0024	0.0205	0.0182	0.0182	0.0098	0.0139	0.0070	0.0071	0.0073	0.0435	0.0568

Nutrient	NO3 ppm					B ppm					SO4 ppm				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	199.39	272.88	190.60	189.56b	251.90b	16.44	17.75	20.31	19.19	23.44	1044	1152a	1923	2227	2017
Fert 2	201.74	207.36	182.67	185.47a	246.98a	17.56	18.06	19.31	20.37	22.81	1293	1579b	2145	2486	2109
Std. Error	1.55	4.60	1.44	1.38	1.41	0.7524	0.6487	0.4791	0.5265	0.7273	142.84	123.06	115.96	107.57	113

Nutrient	Mn ppm					Fe ppm					Cu ppm				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	102	106	187	183 b	205	276	142	343 a	505	280	3.00	3.50 a	5.50	7.50	4.75
80 kg/ha	100	134	173	184 b	182	364	167	478 b	472	320	3.50	4.25 bc	6.00	5.75	5.50
160 kg/ha	108	127	159	164 ab	191	333	201	336 a	450	296	3.50	4.75 c	5.50	6.50	5.75
320 kg/ha	105	114	167	153 a	191	284	163	380 a	488	321	3.50	3.50 a	5.50	4.00	5.50
Fert 2	119	108	172	163 ab	189	292	152	403 ab	486	263	3.50	3.50 a	6.00	5.25	5.25
80 kg/ha	114	108	163	159 ab	196	346	169	348 a	478	312	3.50	3.50 a	6.00	5.25	5.50
160 kg/ha	110	122	175	171ab	182	355	180	389 a	458	284	4.25	3.75 a	5.25	5.50	5.00
320 kg/ha	103	135	166	178 ab	186	378	154	408 ab	549	258	4.25	4.00 ab	6.75	5.25	4.75
Std. Error	8.601	10.540	12.741	8.382	14.381	40.218	14.649	29.025	43.268	24.671	0.2988	0.2537	0.5603	0.9940	0.3134

Nutrient	NO3 ppm					Cl ppm					Zn ppm				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	199.01	213.81a	178.68a	183.5a	251.67a	537.54	557.08a	658.31ab	806.34a	486.15a	20.00	21.75	24.50	23.50	30.25a
80 kg/ha	200.42	241.19b	187.95ab	187.43a	253.43a	538.54	490.78a	577.82a	779.30a	446.87a	24.50	27.25	25.00	24.00	30.25a
160 kg/ha	200.41	280.86c	190.68b	191.32ab	248.77a	578.04	564.13a	656.87ab	817.07a	484.91a	25.25	27.25	23.25	24.75	35.75c
320 kg/ha	197.9	355.65d	203.32c	195.99b	253.73a	553.07	503.91a	653.21ab	732.56a	439.51a	26.75	23.25	21.75	18.75	30.75ab
Fert 2	199.41	207.03a	182.09a	186.4a	245.92a	589.39	516.54a	794.23b	824.63a	396.95a	25.25	24.00	21.75	25.25	31.5ab
80 kg/ha	201.43	208.64a	181.53a	184.21a	244.63a	640.49	758.32a	841.84b	1132.84b	624.63b	25.00	23.50	23.25	23.50	34.25bc
160 kg/ha	200.48	205.28a	184.61a	187.43a	251.82a	587.53	754.61b	1089.17c	1299.68b	709.24b	27.00	26.75	21.75	25.75	31.0ab
320 kg/ha	205.63	208.50a	182.44a	183.82a	245.54a	555.05	954.40c	1289.05d	1541.63c	844.66c	26.75	26.25	24.00	25.25	30.25a
Std. Error	3.0957	9.1962	2.8750	2.7638	2.8206	45.489	51.401	57.923	63.016	36.980	1.412	1.410	1.958	1.943	1.201

Nutrient	Mg %				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Means	0.11875	0.118438	0.12875	0.133125	0.1284
Std. Error	0.0135	0.0112	0.0114	0.0100	0.0083

TABLE V b. Means separation of nutrient contents

Nutrient	TKN				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	6.54	6.94	9.03	8.94 a	9.29 a
80 kg/ha	6.33	7.67	9.84	10.59 b	9.85 ab
160 kg/ha	6.55	7.34	9.47	10.52 b	11.18 bc
320 kg/ha	6.6	7.14	10.76	11.52 b	11.71 c
Std. Error	0.3127	0.3079	0.5927	0.3746	0.5674

Nutrient	SO4				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	0.430	0.474a	0.943	1.151	1.038
Fert 2	0.559	0.653b	1.016	1.300	1.136
Std. Error	0.0565	0.0494	0.0578	0.0642	0.0764

Nutrient	NO3					HPO4					Cl				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	0.088	0.089 a	0.093	0.089	0.137	0.705	0.622	1.144b	1.305	1.255	0.237	0.23 a	0.342a	0.392a	0.264a
80 kg/ha	0.080	0.106 b	0.096	0.101	0.126	0.480	0.699	1.003ab	1.378	0.974	0.214	0.216a	0.297a	0.418a	0.222a
160 kg/ha	0.076	0.110 b	0.088	0.093	0.119	0.541	0.537	0.73 a	1.061	0.905	0.220	0.221a	0.307a	0.398a	0.232a
320 kg/ha	0.085	0.142 c	0.101	0.109	0.133	0.682	0.566	0.827 a	1.096	1.045	0.238	0.201a	0.33 a	0.408a	0.232a
Fert 2	0.079	0.088 a	0.087	0.095	0.140	0.490	0.684	0.883ab	1.301	1.257	0.233	0.219a	0.381ab	0.424a	0.226a
80 kg/ha	0.091	0.087 a	0.087	0.098	0.129	0.630	0.575	0.99 b	1.247	1.188	0.286	0.317b	0.401ab	0.605b	0.328b
160 kg/ha	0.088	0.087 a	0.083	0.097	0.138	0.714	0.569	1.1065 b	1.213	1.065	0.254	0.316b	0.489b	0.668b	0.387bc
320 kg/ha	0.086	0.084 a	0.089	0.098	0.126	0.651	0.600	0.923ab	1.156	1.039	0.234	0.385c	0.632c	0.829c	0.435c
Std. Error	0.0049	0.0052	0.0064	0.0058	0.0083	0.0532	0.0633	0.1002	0.1152	0.1036	0.0206	0.0222	0.0446	0.0500	0.0250

Nutrient	P					K					Ca				
Date	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Mean	0.492	0.481	0.728	0.850	0.941	3.520	3.582	4.272	4.314	4.563	1.275	1.385	1.864	2.132	2.081
Std. Error	0.0613	0.0738	0.1109	0.1114	0.1286	0.4254	0.4380	0.7175	0.5450	0.6242	0.1599	0.1877	0.3113	0.2260	0.2757

Nutrient	Mg					Mn					Fe				
Date	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Std. Error	0.4958	0.4908	0.6259	0.6913	0.6729	0.0453	0.0496	0.0825	0.0882	0.0998	0.0690	0.0689	0.1874	0.2525	0.1523
Mean	0.058	0.062	0.114	0.093	0.079	0.010	0.012	0.016	0.013	0.021	0.035	0.014	0.040	0.048	0.028

Nutrient	Cu					B					Zn				
Date	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Std. Error	0.0015	0.0016	0.0028	0.0029	0.0027	0.0071	0.0740	0.0096	0.0102	0.0121	0.0105	0.0104	0.0112	0.0124	0.0167
Mean	0.0003	0.0002	0.0006	0.0010	0.0004	0.0012	0.0013	0.0014	0.0012	0.0020	0.0015	0.0016	0.0018	0.0027	0.0022

TABLE V c. Means separation of nutrient ratios

Nutrient	P					HPO4					K				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	7.41	6.97	16.84b	18.83c	10.80b	9.32	9.86	23.35b	29.44c	13.89c	51.70	50.99	98.96	94.28b	51.39b
80 kg/ha	7.70	6.72	15.16ab	16.93b	9.8b	8.90	8.30	19.12ab	25.00b	11.38b	55.33	48.84	86.70	82.13a	47.52b
160 kg/ha	7.72	6.55	14.61a	15.48ab	8.09a	9.65	7.74	19.27ab	21.77ab	8.85a	54.16	50.27	85.13	80.91a	40.72a
320 kg/ha	7.92	6.58	13.86a	14.88a	8.28a	10.43	8.29	16.70a	19.52a	9.06a	57.57	48.09	82.90	77.35a	38.96a
Std. Error	0.4078	0.2913	0.6105	0.5841	0.5063	0.7051	0.5450	1.5648	1.4884	0.8178	2.2453	1.7932	4.3904	2.7532	2.2993
Treatment	Ca					Mg					Mn				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	18.13	19.24	43.44b	45.95b	23.61c	7.21	7.11	14.24b	15.00b	7.65c	0.72	0.66	2.01b	1.95b	1.20b
80 kg/ha	20.59	19.70	39.57a	39.57a	20.76b	7.98	6.70	13.03ab	12.99a	6.80b	0.73	0.68	1.69 a	1.73ab	1.03ab
160 kg/ha	19.66	19.29	40.56a	40.56a	19.02ab	7.52	6.71	12.55a	13.33a	6.07a	0.68	0.69	1.61a	1.61a	0.86a
320 kg/ha	20.54	18.16	39.04a	39.04a	17.95a	8.13	6.63	11.74a	12.15a	5.75a	0.68	0.71	1.54a	1.57a	0.85a
Std. Error	0.7409	0.7123	1.5952	1.0783	0.8848	0.3460	0.2701	0.5468	0.4189	0.2439	0.0441	0.0448	0.1003	0.0789	0.0759
Nutrient	Fe					Cu					B				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
0 kg/ha	1.84	0.89	4.21	5.62	1.68b	0.02	0.02	0.06	0.07	0.03b	0.10	0.11	0.23b	0.22b	0.13
80 kg/ha	2.40	0.94	4.14	4.81	1.67b	0.02	0.02	0.06	0.06	0.03b	0.11	0.10	0.20ab	0.18a	0.13
160 kg/ha	2.17	1.04	3.49	4.37	1.35a	0.02	0.02	0.05	0.06	0.02a	0.11	0.10	0.19a	0.21b	0.11
320 kg/ha	2.15	0.89	3.68	4.90	1.30a	0.02	0.02	0.06	0.04	0.02a	0.12	0.10	0.18a	0.18a	0.10
Std. Error	0.2142	0.0525	0.2292	0.3633	0.1109	0.0014	0.0012	0.0045	0.0082	0.0014	0.0076	0.0051	0.0104	0.0080	0.0076
Nutrient	NO3					SO4									
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan					
0 kg/ha	1.29	1.29	2.02b	2.07b	1.52c	7.25	7.06	24.82	28.09b	11.24					
80 kg/ha	1.35	1.27	1.86a	1.87a	1.32b	6.73	7.25	17.45	22.72a	10.84					
160 kg/ha	1.26	1.34	1.81a	1.81a	1.16a	7.69	7.89	20.21	24.24ab	10.00					
320 kg/ha	1.31	1.59	1.78a	1.79a	1.12a	9.10	9.20	19.67	20.65a	10.11					
Std. Error	0.0248	0.0480	0.0521	0.0566	0.0542	1.4013	0.9607	2.1038	1.7203	0.9336					
Nutrient	P					B					SO4				
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	7.51	6.61	15.07	16.38	8.67a	0.11	0.10	0.21	0.19a	0.12	6.95	6.60a	19.69	22.33	9.97
Fert 2	7.86	6.80	15.17	16.69	9.82b	0.11	0.10	0.19	0.21b	0.12	8.44	9.10b	21.38	25.52	11.12
Std. Error	0.2883	0.2060	0.4317	0.4130	0.3580	0.0054	0.0036	0.0074	0.0054	0.0054	0.9909	0.6793	1.4876	1.2164	0.6602
Nutrient	Zn					Cl						NO3	Fe		
Harvest	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan	16-Sep	8-Oct	1-Nov	22-Nov	10-Jan
Fert 1	0.13	0.14	0.28	0.27	0.18b	3.49	3.56ab	7.47bc	8.95ab	2.89bc		1.34b	3.93ab		
80 kg/ha	0.17	0.15	0.25	0.23	0.15a	3.63	2.70a	5.72a	7.60ab	2.14ab		1.32b	4.72b		
160 kg/ha	0.17	0.15	0.23	0.23	0.17ab	3.78	3.12a	6.56ab	7.67ab	2.29ab		1.56c	3.26a		
320 kg/ha	0.17	0.13	0.20	0.18	0.13a	3.53	2.75a	5.89ab	6.93a	1.91a		1.96d	3.51a		
Fert 2	0.16	0.14	0.24	0.28	0.20b	3.77	3.07a	8.67c	9.28b	2.48ab		1.24ab	4.50b		
80 kg/ha	0.17	0.14	0.24	0.25	0.20b	4.26	4.35b	8.57c	11.91c	3.60cd		1.21ab	3.56ab		
160 kg/ha	0.16	0.15	0.21	0.25	0.14a	3.55	4.13b	10.3cd	12.78cd	3.22bc		1.13a	3.72ab		
320 kg/ha	0.18	0.15	0.22	0.24	0.14a	3.68	5.55c	11.93d	14.47d	3.91d		1.21ab	3.85ab		
Std. Error	0.0105	0.0089	0.0233	0.0238	0.0119	0.2911	0.3349	0.5915	0.7208	0.2594		0.0680	0.3242		

Chapter 3

3.0 Fall Fertilization Effects on Douglas-fir Cold Hardiness and Variable Chlorophyll Fluorescence

by

Todd M. Birchler

ABSTRACT. Coastal Douglas-fir (Psuedotsuga menziesii (Mirb.) Franco) 1+1 seedlings were fertilized with two types of fertilizers (NH_4NO_3+K_2SO_4 and $(NH_4)_2SO_4$+KCl) at four rates (0, 80, 160, 320 kg N and K/ha) split over 3 application dates (September 19, October 13, November 1, 1996). Seedling cold hardiness LT_{50} levels on October 23, November 13, and December 9 showed no consistent significant differences among the fertilizer treatments. By December 30 all treatments had attained similar LT_{50} levels (-14 °C). Adding high levels of fertilizers after budset in the fall did not appear to disrupt the cold hardiness process. Levels of chlorophyll fluorescence were affected by the fertilizer types and rates applied. Detectable differences in baseline seedling F_{var}/F_{max} (a quantitative measure of photosynthetic efficiency) among the treatments occurred on November 13 and December 30. Fertilized seedlings had consistently higher F_{var}/F_{max} than unfertilized seedlings. Fall fertilization, if conducted after seedlings have ceased growth and set buds, did not appear to enhance nor detrimentally affect the cold hardening process. This practice may enhance seedling photosynthetic capability which may have positive implications upon outplanting performance.

3.1 Introduction

Cold hardiness is an important physiological adaptation which enables plant tissues to survive exposure to subzero temperatures. In nursery seedlings, cold hardiness is used as an indication of a seedling's ability to tolerate stress. Since reforestation is most successful when seedlings are handled at the time of maximum stress resistance, lifting windows have been established based on the acquisition of cold hardiness (Faulconer 1989).

Seedling nutrient levels, especially nitrogen, may directly impact the seedling hardening process. Too much nitrogen with adequate moisture late in the growing season may prevent seedlings from setting buds and hardening (Hellergren 1981). Conversely, low levels of nutrients during the growing season may result in seedlings of low vigor and subsequent inability to adequately prepare for, or withstand, cold temperatures (DeHayes et al. 1989).

Seedlings are affected by the cold in two ways. Low temperatures affect water viscosity and decrease metabolic and diffusion rates. Freezing causes a reduction of cell water content due to the formation of extracellular ice crystals. Because ice crystals have a lower water potential than cellular water, cellular water moves out of the cell to the ice crystals and the remaining cellular solution concentrates. Damage results when severe dehydration causes changes in membrane properties (Ritchie 1991). Cold hardiness involves physiological changes which allow the avoidance of intracellular freezing and the avoidance or tolerance of freeze-induced dehydration.

When seedling phenology is matched with environmental conditions, the process of hardening normally begins after growth has ceased and next year's buds have formed (in response to decreasing moisture and photoperiod). Physiological changes occur within the seedling in response to declining temperatures which enable the seedling to avoid or tolerate the effects of cold. This involves increasing intracellular solution concentration to lower the freezing point and changing membrane permeability to facilitate the movement of water (Ritchie 1991).

Seedlings unprepared for cold temperatures suffer decreased vigor and perhaps death. In the nursery, a sudden cold snap before seedlings are sufficiently hardened can decimate entire crops. In the field, seedlings which break bud early may be susceptible to late spring frosts. It is therefore of interest to examine how various physiological factors, such as seedling mineral nutrition, relate to the cold hardening process. However, it is difficult to link a particular seedling attribute directly with seedling cold hardiness due to the many interrelating factors (i.e. seedling water status, carbohydrate status). Seedling physiology and the hardening process can be altered at many points and by various environmental and/or nursery cultural mechanisms such as the lengthening of the growing season to attain larger seedlings through fertilizer and irrigation inputs. This may not leave sufficient time for the seedlings to set bud and begin the hardening process before the onset of cold temperatures.

3.1.1 Literature Review

Previous research has examined the relationship between seedling nutrition and hardening. Generally, seedlings were cultured to different nutrient levels then subjected to a particular freezing temperature in a liquid bath (Christersson 1973, Pellett 1973, Hellergren 1981) or whole plant freeze test (Timmis 1974) followed by visual assessment, or were evaluated by the electrolyte leakage method (Aronsson 1980, Columbo et al. 1984). In many instances seedlings were tested before any cold acclimatization occurred or after a specified exposure to declining photoperiod and temperatures. Freeze testing was typically conducted only once during the fall or winter.

Seedling cold hardiness development is a dynamic and integrated response to the environment. Therefore, relating seedling mineral nutrition with cold hardiness must consider the effects of photoperiod, moisture, and temperature. For example, increases in nitrogen concentrations as a result of fertilizers applied conventionally during the growing season (Christersson 1973, Hellergren 1981) or before a seedling begins to harden (DeHayes et al. 1989) may have different effects on cold hardiness than when fertilizers are applied (or withdrawn) during or after seedlings begin to harden (Timmis 1974). Few

studies have examined the effects of fall fertilization on seedling cold hardiness (Thompson 1983, Gleason et al. 1990).

Results of studies of nitrogen and potassium nutrition on cold hardiness are variable. Aronsson (1980) reported increased cold hardiness in Scots pine (*Pinus sylvestris* L.) seedlings containing 1.3 to 1.8% nitrogen as compared to seedlings containing nitrogen outside of this range. However, in another study, Scots pine seedlings with 1.7% nitrogen exhibited slightly lower cold hardiness than seedlings with 0.84 to 1.4% nitrogen (Hellergren 1981). Nitrogen fertilization in the mid- and late summer resulted in hardier red spruce (*Picea mariana* Mill. B.S.P.) seedlings (DeHayes et al. 1989). The seedlings receiving nitrogen acclimated to the cold more rapidly in the fall and deaclimated more rapidly in the spring. Supplemental phosphorus was not linked with cold hardiness in red spruce seedlings (DeHayes et al. 1989), but was indirectly related to frost damage in Sitka spruce (*Picea sitchensis* Bong. Carr.) seedlings because it resulted in the extension of the growing season into frost prone times of the year (Malcolm and Freezaillah 1975). Douglas-fir seedlings fertilized with varying levels of nitrogen (20 to 250 ppm) and phosphorus (4 to 60 ppm) differed in nitrogen concentrations (1.2 to 2.6%) but no consistent differences in cold hardiness resulted (Hawkins et al. 1995).

Potassium fertilization (as KCl) is believed to improve cold hardiness in white spruce (*Picea glauca* (Moench.)Voss.), white pine (*Pinus strobus* L.), and red pine (*Pinus resinosa* Ait.) seedlings, although there were not corresponding increases in foliar potassium levels as a result of KCl fertilization (Kopitke 1941). Conversely, potassium increased in Scots pine seedlings but cold hardiness improvements were minimal (Christersson 1973), or nonexistent (Aronsson 1980). Similarly, (Edwards 1989) found only a weak correlation between cold hardiness and nutrient concentrations in containerized lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Englem.), jack pine (*Pinus banksiana* Lamb.), red pine, Scots pine, and white and black spruce seedlings cultured under hardening regimes of various N, P, and K levels. Shoot concentrations of N, P, K did not correlate with survival at -5 and -10° C. Of the N/P, N/K, and P/K ratios examined, only the N/P ratio correlated with survival and only for Scots pine. KCl applied to *Festuca arundinaceae* in the fall was not critical to the development of freezing

tolerance (Cook and Duff 1976), and when applied to the ornamental shrub, *Forsythia x intermedia* Zab. "Lynwood", freezing injury increased significantly as potassium concentration increased from 1.51 to 4.12 % (Beattie and Flint 1973). Nitrogen was decreased as potassium increased. Timmis (1974) found the balance between nitrogen and potassium was more closely related to cold hardiness than their respective levels in containerized Douglas-fir (*Psuedotsuga menziesii* (Mirb.)Franco.) seedlings.

While balanced mineral nutrition is important for maintaining vigorous seedling physiology, the relationship between nutrient levels and cold hardiness is less clear. Increased levels of nutrition may result in improved outplanting performance (Jopson and Paul 1984, Margolis and Waring 1986, van den Driessche 1988), therefore practices to attain higher levels of nutrients have been employed. Fall fertilization is one alternative for culturing seedlings with above normal levels of nutrition (nutrient loading). Fall fertilization regimes are easily implemented in both bareroot and containerized nurseries by simply applying nutrients in addition to the conventional fertilizers applied during the growing season, after seedling growth has ceased and buds have set. Thus it is of interest to examine the cold hardening process in fall fertilized Douglas-fir seedlings in order to better evaluate this practice.

Variable chlorophyll fluorescence has emerged as a tool to evalutate seedling response to freezing stress (Lindgren and Hällgren 1993, Strand and Öquist 1988). Variable chlorophyll fluorescence is a method which can rapidly and nondestructively evaluate the physiological status of a plant (Vidaver et al. 1989, Vidaver et al. 1991). The fluorescence emitted from a dark adapted seedling is measured when exposed to saturating light intensities. Light energy absorbed by a leaf can either be dissipated as heat (75-97%), utilized for photochemistry (0-20%), or reemitted as far red fluorescence (3-5%) (Vidaver et al. 1991). Since these processes are competitive, any change in the photosynthetic rate will necessarily produce corresponding changes in fluorescence emission.

Fluorescence emitted by a dark pretreated seedling follows a characteristic curve (Figure 18). This Kautsky curve is composed of a rapid kinetics (0-1s) and slow kinetics (1-300s) phase. Initially, there is a rapid rise in fluorescence to a level (F_o) which

represents fluorescence when most of the photosystem II (PSII) reaction centers are fully open and the primary electron acceptor (Q_A) is fully oxidized and resistance to electron energy flow is minimal (Vidaver et al. 1991). This ground level fluorescence represents processes independent of photochemistry and is used to standardize subsequent fluorescence emissions since it is correlated with shoot size (Vidaver et al. 1991). Fluorescence increases to a maximum (F_{max}) as the Q_A pool becomes increasingly reduced and the water splitting portion of photochemistry catches up with electron transport (Vidaver et al. 1991). Fluorescence declines during the slow kinetics phase as electrons are transported away from Q_A thus maintaining Q_A in a reduced state and "quenching" fluorescence. Terminal or steady state fluorescence (F_t) is reached in 1-5 minutes and the reduction in fluorescence from F_{max} to F_t is a result of photochemical and nonphotochemical quenching of fluorescence. At steady state, photochemistry is in balance with with carbon assimilation (Bolhàr-Nordenkampf et al. 1989). Variable fluorescence (F_{var}) is the difference between F_{max} and F_o. The ratio F_{var}/F_{max} is a quantitative measure of photochemical efficiency (Mohammed et al. 1995) and is linearly correlated with the quantum yield (quantum yield=yield of photochemical products/number of quanta absorbed (Taiz and Zeiger 1991)) of photosynthesis (Bolhár-Nordenkampf et al. 1989). Thus, any factor that affects the process of photosynthesis will produce changes in fluorescence.

F_{max} is affected by length of the dark pretreatment, level of excitation light energy, and the relative stage of vegetative development (Vidaver et al. 1991). F_o can be affected by environmental conditions or stresses which cause structural changes at the chlorophyll pigment level of PSII (Krause and Weis 1984). Thus many factors impact the F_{var}/F_{max} ratio. Informed interpretation of fluorescence curves and ratios not only requires baseline data with which to make comparisons and an understanding of the seedling's past cultural history, but also strict sampling protocol.

To illustrate some of the complexities involved in the interpretation of fluorescence emissions a general discussion of the possible significances of high and low fluorescence is

presented below. A high fluorescence emission may be due to efficient light harvesting accessory pigments of PSII funneling light energy to the PSII reaction center causing the ejection of more electrons than can be accepted and transported along the electron transport mechanism. The excess energy not captured by the electron transport mechanism will increase both the heat given off and the fluorescence emitted. Thus, a high fluorescence may be indicative of a very efficient light harvesting/ reaction center complex. A high fluorescence may also be an indication of a disruption of the electron transport mechanism which would prevent the transport of electrons from the PSII reaction center. This would cause a buildup of energy and also result in high fluorescence (and heat) emissions. This second scenario is initially readily identified because the relative fluorescence does not decrease from its maximum as would normally be the case.

A low fluorescence emission may indicate an inefficient light harvesting complex as a result of environmental stress and/or a slowing of physiological processes. Thus, there is not enough energy reaching the reaction center and as a result there is plenty of oxidized primary electron acceptors (Q_A) for the number of electrons being ejected from the reaction centers.

Variable chlorophyll fluorescence has been used to examine dormancy induction, photochemical inactivation, frost hardiness, and freezing damage in several species, most notably such boreal conifers as Scots pine, lodgepole pine, red spruce, Norway spruce *(Picea abies* (L.) Karsten), black sprue (*Picea rubens* Sarg.), and white spruce (Strand and Öquist 1988, Vidaver et al. 1988, Vidaver et al. 1989, Lindgren and Hällgren 1993, Devisscher et al. 1995, Binder and Fielder 1996) and to some extent for the more temperate Douglas-fir (Hawkins and Lister 1985, Fisker et al. 1995).

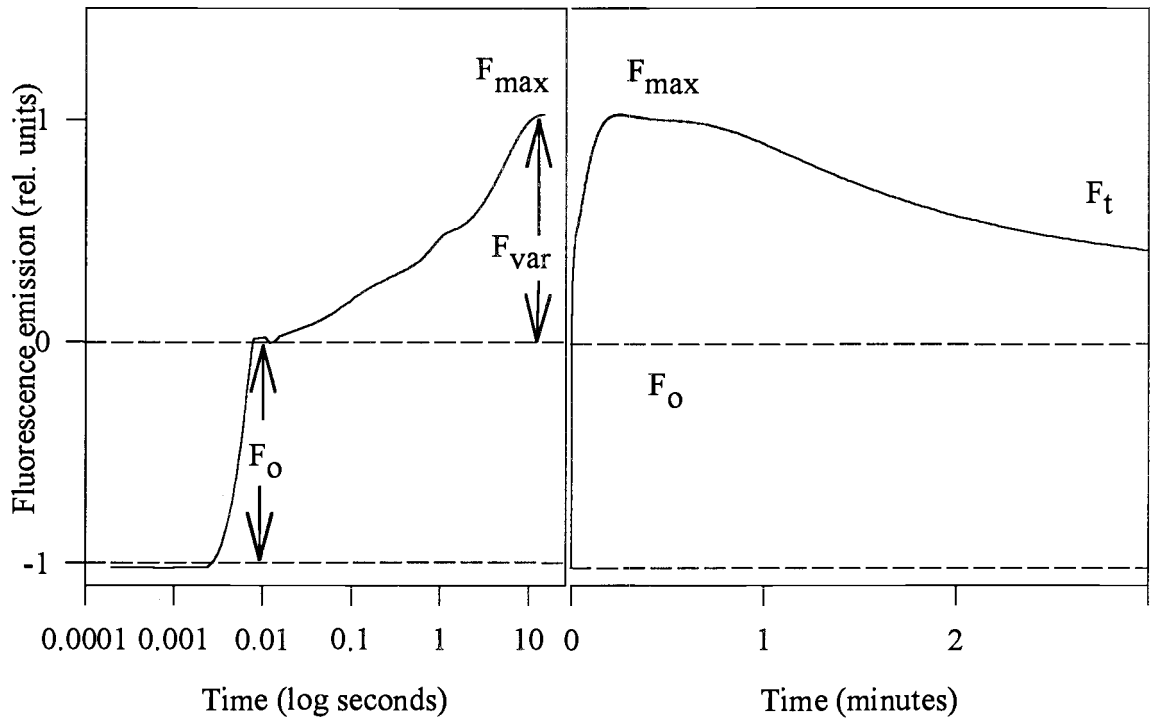


FIGURE 18. Fluorescence induction (Kautsky curve) parameters and definitions. Ground fluorescence (F_o) occurs when the electron acceptors are fully oxidized. Maximum fluorescence (F_{max}) occurs when the electron acceptors are fully reduced. Variable fluorescence (F_{var}) is the difference between maximum and ground fluorescence (F_{max}-F_{var}). Terminal fluorescence (F_t) is reached after several minutes as photochemistry and carbon assimilation reach a steady state. Adapted from Vidaver et al. (1991).

Variable chlorophyll fluorescence has also shown promise for detecting copper and phosphorus deficiency in Monterey pine (*Pinus radiata* D. Don.) (López Gorgé et al. 1985, Conroy et al. 1986) and Douglas-fir (Vidaver et al. 1988) seedlings. In this study, variable chlorophyll fluorescence is used to evaluate seedling response to freeze treatments and fall fertilization.

3.1.2 Objectives and Questions of Interest

The objectives of this study were to examine the cold hardiness process in nitrogen and potassium fall-fertilized Douglas-fir seedlings. Cold hardiness was examined at four different dates using the whole plant freeze test followed by variable chlorophyll fluorescence and visual assessments. An examination of how baseline variable chlorophyll fluorescence reflects changes in the nutritional status of Douglas-fir was conducted. Also, an examination of the usefulness of variable chlorophyll fluorescence for assessing freeze-treated seedling vitality was conducted on unfertilized seedlings.

Null hypotheses tested were: fall fertilization with four rates of NH_4NO_3+K_2SO_4 or $(NH_4)_2SO_4$+KCl does not affect:

1. seedling cold hardiness LT_{50} levels at each date
2. baseline chlorophyll fluorescence F_{var}/F_{max} levels

and variable chlorophyll fluorescence F_{var}/F_{max} of baseline and freeze-treated seedlings do not correlate with seedling damage or mortality in unfertilized seedlings.

3.2 Materials and Methods

3.2.1 Nursery Stock

Two year old (1+1) bareroot Douglas-fir seedlings from a coastal Oregon seed source (M417295 071C12, elev. 1500 ft) were grown under standard nursery cultural practices at the D.L. Phipps State Forest Nursery located 5 km south of Elkton, Oregon. Seedlings were sown in March 1995, lifted, pruned, and transplanted during October, 1995 at a

density of approximately 12.5 seedlings per linear meter of nursery bed (74 seedlings per square meter). After transplanting, but before the initiation of this study, the seedlings had received approximately 126 kg N/ha, 8 kg P/ha, 15 kg K/ha, and 27 kg S/ha along with micronutrients applied over four applications between late March and early June. Seedlings were topmown in July, wrenched in early September, and drought stressed for 2 weeks prior to the initiation of this study to induce bud set and the onset of dormancy. Treatments for this study began on September 19, 1996.

3.2.2 Fertilizer Application

The treatments consisted of two types of fertilizers ($(NH_4NO_3+K_2SO_4$ and $(NH_4)_2SO_4+KCl$) at four rates (0, 80, 160, 320 kg/ha) of total nitrogen and potassium divided over three application dates (i.e. the first application for the 80 kg/ha rate would be 80/3 or 26.7 kg/ha). Three equal applications were implemented to maximize the duration of the nutrients in the rooting zone since all of the included cations and anions are potentially readily leachable from the soil profile and typical fall and winter precipitation in the Pacific Northwest is quite high.

Fertilizers were applied on September 19, October 11, and November 1 using a CO_2 powered applicator constructed of 1/2 inch PVC tubing. The applicator had six nozzles designed to apply fertilizers between the rows of seedlings as close to the ground as possible. Due to varying distance of each nozzle from the pressure source, differing spray velocities emitted by the nozzles were corrected by utilizing nozzles of three different apertures to achieve relatively uniform spray quantities. The fertilizers were added to water in a three gallon tank to allow for good dissolution of the fertilizers and sufficient multiple passes by the applicators to ensure uniform application. Premixed concentrated solutions were filtered into the spray tank and any resulting residue was redissolved two to three times thus ensuring the maximum dissolution of the fertilizers.

3.2.3 Cold Hardiness

3.2.3.1 Freeze Testing

Seedlings were tested for cold hardiness using the whole plant freeze test. Seedlings were sampled from each treatment plot for cold hardiness using a randomly predetermined sampling format. Samples were harvested on October 23, November 13, December 9, and December 30, 1996. A shovel was used to cut around all sides of the group of sample seedlings and the seedlings were carefully lifted from the seedbed, placed into coolers, transported to Oregon State University, and stored overnight at 5.5° C. The seedlings were tagged and potted into one gallon containers of a peat and vermiculite mixture. Four seedlings were potted into each container, one from each block of the same treatment. (Note: this caused a restriction on the randomization of seedlings within the freezer, but was necessary in order to evaluate the seedlings using variable chlorophyll fluorescence). After potting, the seedlings were watered and placed outside until freezer treatment. The pots were randomly placed into the freezer and since only one freezer was available, psuedoreplication of the freeze treatments occurred. Four temperatures were chosen based on their expected ability to bracket the LT_{50}. Thus, four days were necessary to conduct the freeze treatments.

A programmable chest freezer (Kenmore 23) with an 18-pot foam insulating mold was utilized for the freeze treatments. This freezer contained a thermostatically controlled heating unit on one end so the freezer temperature oscillated one degree above and below the target temperature. Temperatures were lowered from room temperature to 0°C at 20°C per hour, then decreased to the target temperature at 5°C per hour, held at the target temperature for 2 hours, then raised back to 0°C at 20°C per hour (Tanaka et al. 1995). Seedlings were removed and placed into a growth room with adequate moisture, ambient photoperiod, and temperatures between 15.5 and 26.7° C.

3.2.3.2 Visual Assessment

Seven days after freeze treatments, seedlings were removed from the growth rooms and visually assessed for damage. Foliar damage was estimated as a percentage of total seedling foliage which were brown or dried. Ten randomly selected buds from the entire length of seedling shoot were longitudinally sectioned and examined for evidence of browning. Finally, the outer bark of the stem over the entire length of the shoot was scraped with a razor blade to examine for evidence of cambial damage. Cambial damage is manifested through varying degrees of browning, which is in sharp contrast to the vibrant green of live cambial tissue. Determining seedling viability must integrate several tissues and take into account tissue location as well as degree of damage. The first consideration is the cambial tissue. If the cambium is dead below the lowest bud, then obviously the seedling is nonviable. If the middle or top cambium is dead, then whether a seedling is viable or nonviable depends on the number of live buds. If greater than 50% of the buds are damaged, then the seedling is considered nonviable. The foliage only becomes a determining factor when cambium or bud damage is borderline (Tanaka et al. 1995, Fisker et al. 1995). Since viability does not mean vigor, the long term growth and production of seedlings determined as viable, but that exhibit damage, will naturally be affected.

The LT_{50} was determined by plotting percent survival against temperature, and assuming a straight line relationship, the LT_{50} was the temperature corresponding to the point on the line where 50% of the seedlings are estimated to have been killed.

3.2.4 Variable Chlorophyll Fluorescence Assessment

3.2.4.1 Baseline Fluorescence

One day after potting the seedlings, one seedling from each treatment replicate were assessed for chlorophyll fluorescence. Seedlings were exposed to a three hour light pretreatment in a specially designed and ventilated light box, followed by a dark pretreatment of one half hour. The light pretreatment (of approximately 100μE) was to

standardize the environment of all the seedlings. The dark pretreatment is to ensure that all of the electron carriers are fully oxidized and able to accept and transfer electrons when illuminated (Vidaver et al. 1991). Fluorescence was measured using an integrating fluorometer (Pacific Fluorotec, Burnby, B.C.) which enables measurement of a large portion of the shoot.

After the dark treatment, seedlings were transferred into the fluorometer's sphere. Complete darkness was maintained in the room. Attempts were made to ensure that the same portion of the shoot was assessed on all seedlings. Each seedling was illuminated for three minutes during which chlorophyll fluorescence emissions were recorded (FluoroView 0.5d, 1991). Fluorescence measurements were conducted between 9 am and 2 pm.

3.2.4.2 Fluorescence of Freeze Treated Seedlings

One day after freeze treatment, one seedling from each of the 32 treatment replicates was assessed for chlorophyll fluorescence as described above.

3.3 Experimental Design and Statistical Analysis

The experimental design for the application of fertilizers was a randomized complete block design with four blocks and a 2x4 factorial (two fertilizer types and four rates). The eight treatments were randomly assigned to a 6 m length of nursery bed on each of four nursery beds (blocks or replications).

The LT_{50}'s were determined for each replicate of each treatment using four freezer temperatures to bracket the LT_{50}. At each of the four freezer temperatures the mean seedling vitality for three trees was recorded for each replicate. The LT_{50} for each replicate was determined assuming a straight line relationship between freezer temperature and seedling vitality.

Analysis of variance (ANOVA) was utilized to examine the LT_{50}'s as a result of the fertilizer type, fertilizer rate, and type x rate. Significant differences among means were determined using Fisher's Protected Least Significant Difference at a 0.05 level of

significance (Steel and Torrie 1980). The assumptions of normality, linearity, and constant variance were verified by examination of the residuals but no transformations were necessary. Correlation coefficients between F_{var}/F_{max}, seedling vitality, and needle and bud damage were determined.

The repeated measures of baseline fluorescence over time were analyzed using multivariate analysis of variance (MANOVA). Significant multivariate tests of baseline F_{var}/F_{max} were based on Wilks' criteria (Johnson and Wichern 1988). Statistical Analysis Software (SAS Institute 1989) was utilized for all data analysis.

Correlation coefficients between F_{var}/F_{max}, seedling vitality, and needle and bud damage were determined to examine the relationship between variable chlorophyll fluorescence of baseline and freeze-treated seedlings unfertilized seedlings and seedling damage and mortality. The F_{var}/F_{max} at the LT_{50} ($LT_{50}F_{var}/F_{max}$) was determined from the equation:

$$LT_{50}F_{var}/F_{max} = Y_1 + ((Y_2-Y_1)/(X_2-X_1)) * (LT_{50} - X_1)$$

where X_1 and X_2 are the two temperatures bracketing the LT_{50} and Y_1 and Y_2 are the mean F_{var}/F_{max} at the two temperatures (Fisker et al. 1995).

3.4 Results

3.4.1 General Results

Fall fertilization with NH_4NO_3+K_2SO_4 or $(NH_4)_2SO_4$+KCl did not alter the Douglas-fir cold hardiness process. There were no differences among treatments on mean LT_{50}'s on the October 23 harvest prior to hardening. During the November 13 and December 9 harvests there were detectable, though not consistent nor biologically significant differences among the treatments. By December 30, all seedlings had attained the same level of cold hardiness. The hypothesis of no treatment effect on baseline fluorescence

was rejected. There was a significant fertilizer type and fertilizer rate effect on mean F_{var}/F_{max} values on November 13 and a significant fertilizer rate effect on December 30. On these dates, seedlings fertilized with NH_4NO_3+K_2SO_4 or $(NH_4)_2SO_4$+KCl had significantly increased F_{var}/F_{max} over unfertilized seedlings. Although not significant on December 9, the same trend was evident.

The relationship between F_{var}/F_{max} and needle damage was generally strong (Table 1), but the relationship between F_{var}/F_{max} and seedling vitality was more variable. Seedling $LT_{50}F_{var}/F_{max}$ declined as seedling LT_{50} declined.

3.4.2 Cold Hardiness

Less than 20 percent of the seedlings from all treatments survived the -2° C freezer treatment during the October cold hardiness assessment. As a result, the LT_{50} could not be determined. There were significant fertilizer type x rate interaction effects among mean seedling LT_{50}'s during the November 13 and December 9 cold hardiness assessments (P=0.0002, 0.0184) although there were no consistent pattern to these differences (Figure 19). Most treatments became more cold hardy during the fall although one treatment, the 80 kg/ha $(NH_4)_2SO_4$+KCl , actually became less cold hardy from November 13 to December 9 (-8.3 to -6.7° C). By December 30, all treatments had attained similar LT_{50}'s with a mean of -13.7° C (Figure 19).

3.4.3 Variable Chlorophyll Fluorescence

3.4.3.1 Baseline Fluorescence

While there were no differences in mean F_{var}/F_{max} values among the treatments during the October evaluations, in November seedlings fertilized with $(NH_4)_2SO_4$+KCl had significantly higher F_{var}/F_{max} (0.5572) than seedlings fertilized with NH_4NO_3+K_2SO_4 (0.5285, P=0.0182, Figure 20).

Unfertilized seedlings had a significantly lower F_{var}/F_{max} (0.4979) than did the fertilized seedlings at the 80, 160, and 320 kg N/ha rates (0.5612, 0.5752, 0.5371, respectively, P=0.0005, Figure 20). Among the fertilized seedlings, the 80 and 160 kg/ha treatments had the highest mean F_{var}/F_{max}.

In January, the unfertilized seedlings again had a significantly lower F_{var}/F_{max} (0.4882) and the 320 kg/ha treatment had the highest (0.5495, P=0.0477). As expected, there was a trend over time although this trend was not affected by the fertilizer treatments. Mean F_{var}/F_{max} increased from October to December, but declined from the December to January (P=0.0001). Kaustky curves of relative fluorescence illustrate the detectable differences among fertilizer types and rates during November and fertilizer rates in January (Figures 21 and 22). Fertilized seedlings consistently had higher levels of fluorescence during the three minute scan (Figure 21). Seedlings fertilized with $(NH_4)_2SO_4$+KCl had higher F_{max} values but generally slightly lower fluorescence during the quenching phase (Figure 22).

3.4.3.2 Fluorescence of Freeze Treated Seedlings

As expected, F_{var}/F_{max} ratios correlated well with seedling foliar damage (r=-0.81 to -0.86) with the exception of the October date. The correlation of F_{var}/F_{max} with seedling vitality was more variable (r=0.15 to 0.71, Table 4). On some dates, F_{var}/F_{max} was better able to detect nonviable seedlings (November 13, December 30) than on others (October 23, December 9). Also, F_{var}/F_{max} correlated marginally well with bud damage (r=-0.44 to -0.87).

Seedling $LT_{50}F_{var}/F_{max}$ of the unfertilized seedlings paralleled the declining LT_{50} from November to January (Figure 23). Seedling $LT_{50}F_{var}/F_{max}$ declined from 0.4165 in November to 0.2008 in January concurrent with the LT_{50} decline from approximately -8° to -14°C.

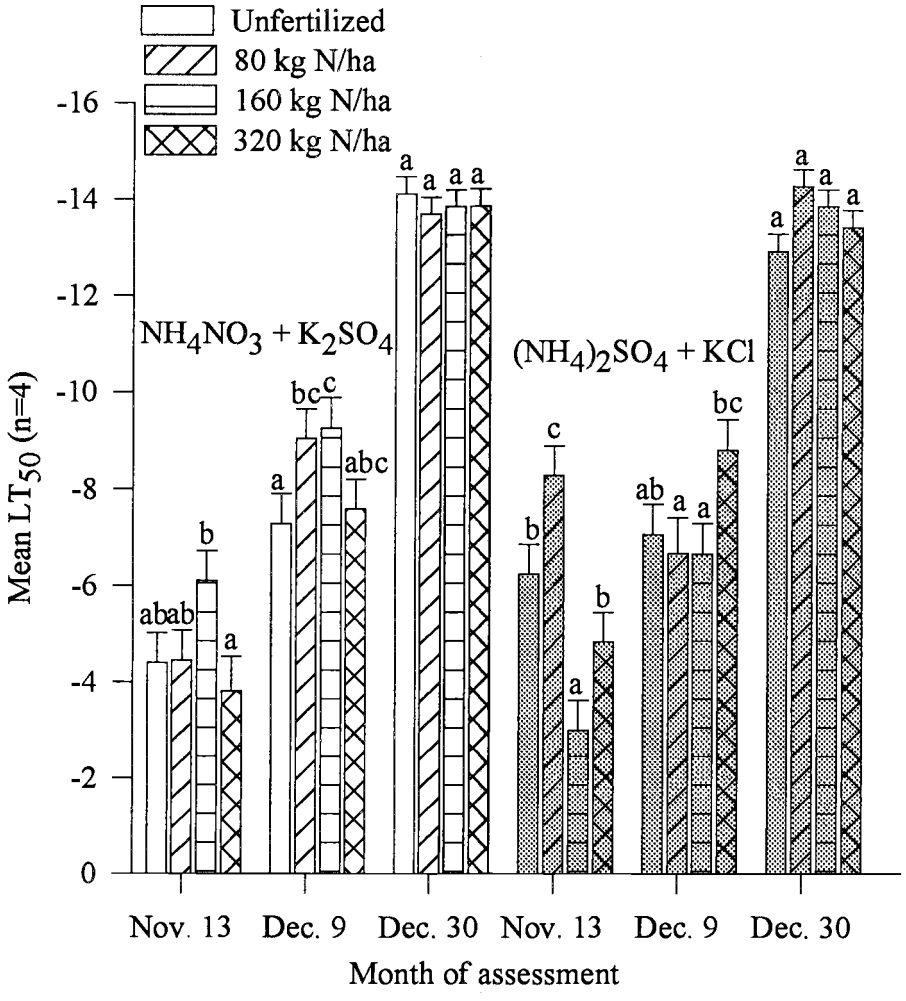


FIGURE 19. Mean seedling LT_{50} by treatment and month. Means followed by different letters within each month are significant at P=0.05.

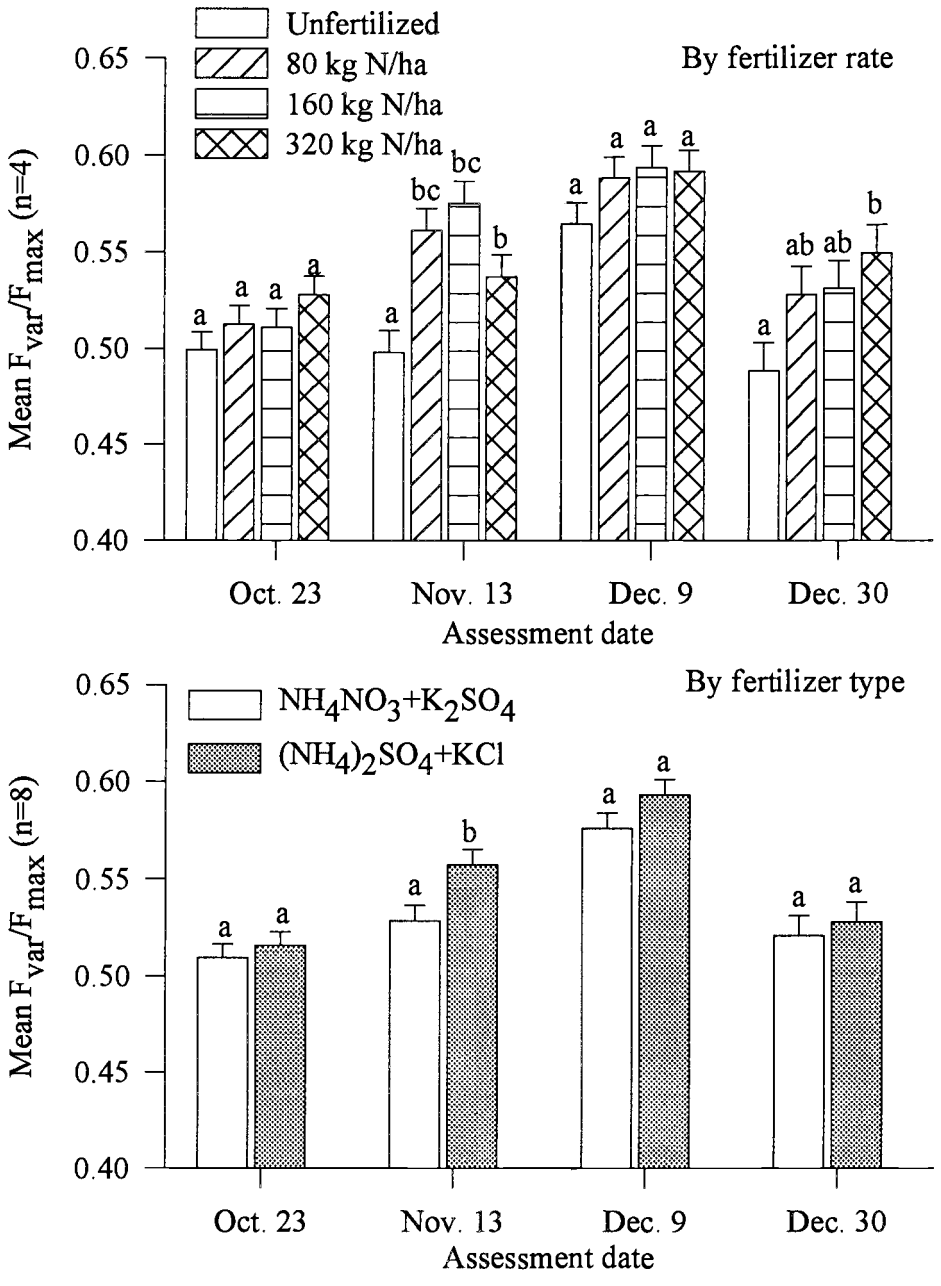


FIGURE 20. Mean seedling baseline F_{var}/F_{max} ratios over time by fertilizer rate and type. Means followed by different letters within each month are significant at P=0.05.

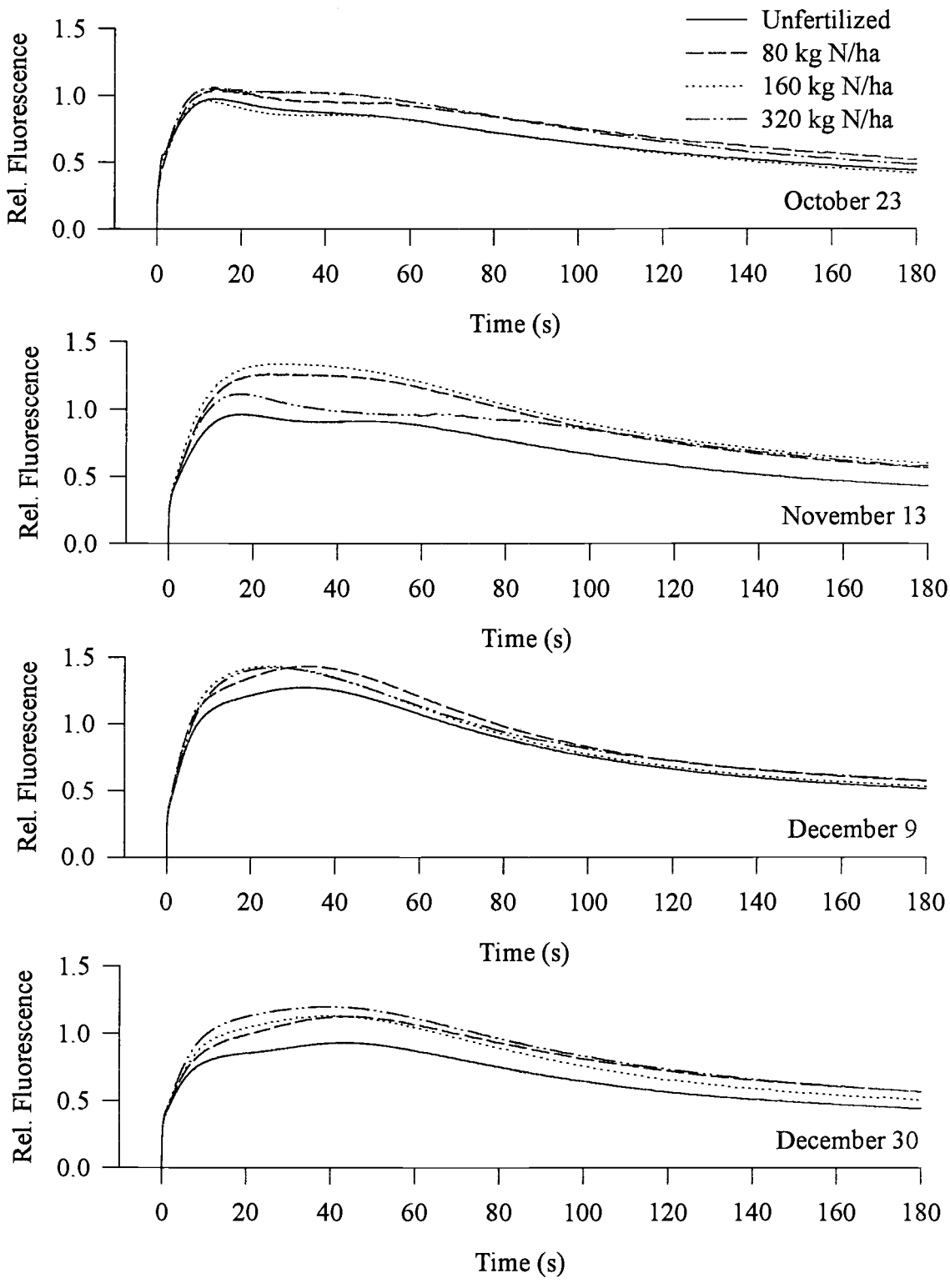


FIGURE 21. Relative fluorescence by fertilizer rate for each assessment date. Each line is the mean of 8 scans.

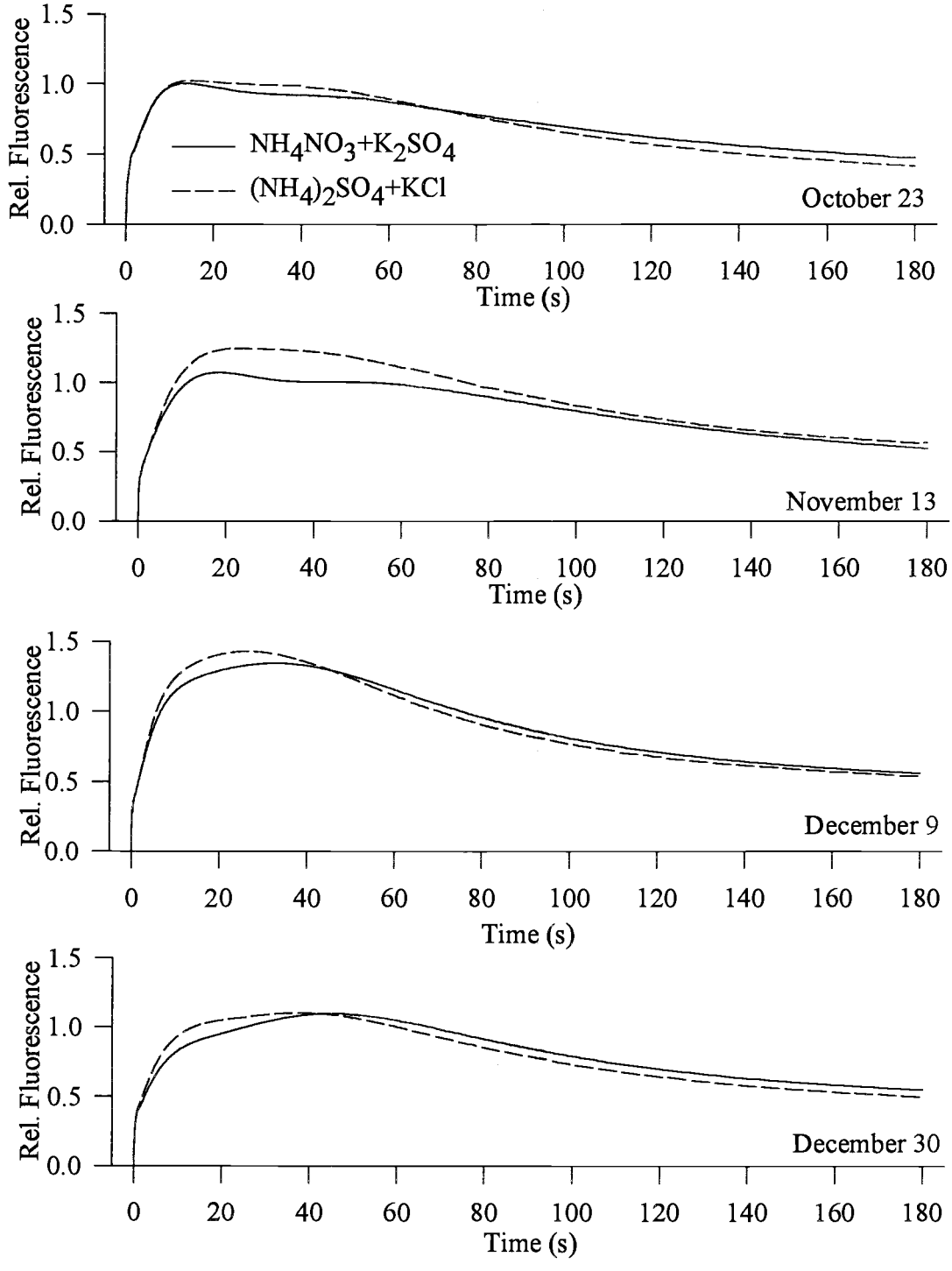


FIGURE 22. Relative fluorescence by fertilizer type and assessment date. Each line is the mean of 16 scans.

TABLE 4. Pearson's correlation coefficient comparing several seedling cold hardiness parameters.

Date	Variable	Needle	Vitality	Fv/Fm	Dead buds
October	Needle	***	-0.378	-0.309	0.672
	Vitality	-0.378	***	0.153	-0.209
	Fv/Fm	-0.309	0.152	***	-0.441
	Dead buds	0.672	-0.209	-0.441	***
November	Needle	***	-0.698	-0.857	0.842
	Vitality	-0.698	***	0.691	-0.662
	Fv/Fm	-0.857	0.691	***	-0.875
	Dead buds	0.843	-0.662	-0.875	***
December	Needle	***	-0.402	-0.824	0.635
	Vitality	-0.402	***	0.167	-0.114
	Fv/Fm	-0.824	0.167	***	-0.673
	Dead buds	0.635	-0.114	-0.673	***
January	Needle	***	-0.537	-0.808	0.587
	Vitality	-0.537	***	0.708	-0.952
	Fv/Fm	-0.808	0.708	***	-0.759
	Dead buds	0.587	-0.952	-0.759	***

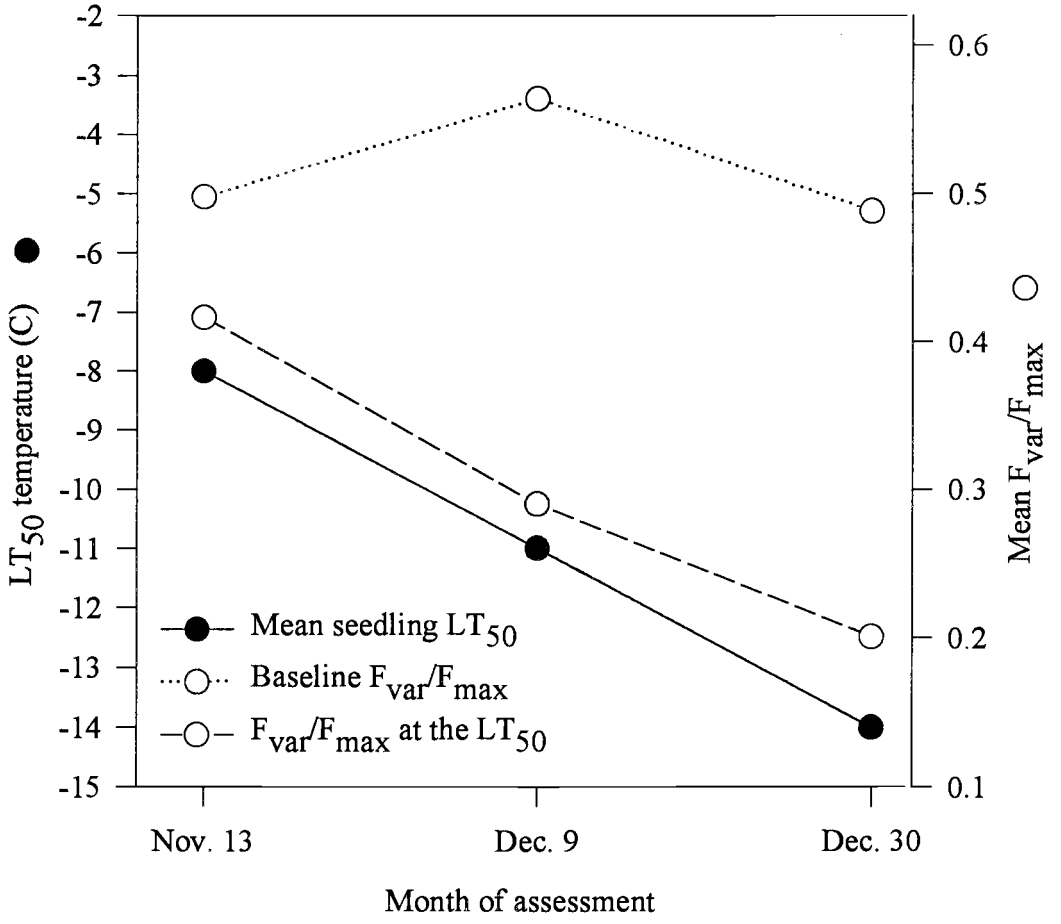


FIGURE 23. Mean seedling LT_{50}, F_{var}/F_{max} of frozen seedlings, and baseline F_{var}/F_{max} from November 13 to December 30 of the unfertilized seedlings.

The increased F_{var}/F_{max} observed in the fertilized baseline seedlings did not appear to have a consistent effect on freeze-treated seedling F_{var}/F_{max}. There were no consistent significant effects observed (Table 5).

3.5 Discussion

3.5.1 General Discussion

Fertilizing coastal Douglas-fir seedlings with as much as 320 kg N/ha split over several applications after buds have set and dormancy induced does not appear to affect the cold hardening process. All treatments acheived the same level of hardiness by January. Differences in F_{var}/F_{max} were detectable on two of the four sample dates. Seedling $LT_{50}F_{var}/F_{max}$ of unfertilized seedlings declined as the LT_{50} declined.

3.5.2 Cold Hardiness

Although these cold hardiness results are not conclusive, it does appear that fall fertilization-induced foliar TKN increases (from 1.5 to 2.2%) does not impede the seedling hardening process. There were statistically significant differences among the treatments on two of the four harvest dates. However, because these results were not consistent, their biological significance is questionable. The LT_{50}'s were determined using only a subsample of three seedlings from each treatment plot. Thus, the erratic LT_{50} results obtained for November and December may reflect not only wide variation during initial hardening, but also the small subsample number. Also, four days were necessary to conduct the freezer treatments. Seedlings were kept outside in pots during this time but some dehardening may have occurred during the potting and holding process.

TABLE 5. ANOVA P-values of F_{var}/F_{max} values of freeze-treated but unfertilized seedlings by treatment over time.

Date	Effect	-2	-4	-6	-8
23-Oct	Block	0.3047	0.3869	0.1770	0.1522
	Fert	0.4874	0.0001	0.0002	0.1000
	Rate	0.0305	0.0366	0.0001	0.1408
	FXR	0.9067	0.0121	0.0116	0.0526
Date	Effect	-1	-4	-7	-10
13-Nov	Block	0.7440	0.4298	0.6369	0.7455
	Fert	0.1538	0.0179	0.3240	0.7738
	Rate	0.1877	0.0586	0.9371	0.0891
	FXR	0.0854	0.0493	0.0915	0.0297
Date	Effect	-4	-7	-10	-12
9-Dec	Block	0.5778	0.3253	0.0401	0.4575
	Fert	0.1267	0.3685	0.4079	0.3906
	Rate	0.3212	0.0488	0.1312	0.1423
	FXR	0.3045	0.1977	0.4131	0.0577
Date	Effect	-4	-8	-12	-16
30-Dec	Block	0.7689	0.9209	0.3297	0.6636
	Fert	0.7108	0.1742	0.0643	0.0052
	Rate	0.3533	0.5889	0.0123	0.0001
	FXR	0.9801	0.4429	0.0144	0.0064

Of importance is the similarities in seedling LT_{50}'s among the treatments by January. All seedlings achieved an LT_{50} of approximately -14° C with little variation. An LT_{50} of -15° C has been considered a target for coastal Douglas-fir seedlings in that seedlings may be safely lifted and prepared for outplanting (Faulconer 1988). Thus, applying up to 320 kg N and K/ha in the fall in three applications, after dormancy has been induced, did not change the target LT_{50} of these Douglas-fir seedlings. These results differ from those which found improved cold hardiness in fall fertilized Douglas-fir and ponderosa pine (Thompson 1985, Gleason et al. 1991). However, these tests were conducted only once. Similar to the results of Hawkins et al. (1995) who tested Douglas-fir seedlings containing 1.2 to 2.6% nitrogen, there was no apparent correlation between foliar nitrogen level and cold hardiness such as was observed for Scots pine (Aronsson 1980, Hellergren 1981). A decrease in cold hardiness was observed when nitrogen concentrations were greater than 1.7 and 1.8%.

3.5.3 Variable Chlorophyll Fluorescence

3.5.3.1 Baseline Fluorescence

Variable chlorophyll fluorescence was able to detect differences as a result of fertilization. I am not aware of any studies which have examined the relationship of variable chlorophyll fluorescence F_{var}/F_{max} and seedling nitrogen levels. Seedlings fertilized with nitrogen had consistently higher F_{var}/F_{max} and the overall Kautsky curves show this (Figure 21). Seedlings fertilized with $(NH_4)_2SO_4$+KCl had consisently higher F_{var}/F_{max} than seedlings fertilized with NH_4NO_3+K_2SO_4 (Figure 22). Other studies have shown the ability of variable chlorophyll fluorescence to detect nutrient (specifically copper and phosphorus) deficiency in Douglas-fir (Vidaver et al. 1988) and Monterey pine (Lopez Gorge et al. 1985, Conroy et al. 1986). Also, the general trends between the two fertilizer types are interesting as well. Seedlings fertilized with $(NH_4)_2SO_4$+KCl received twice as much sulfate (208, 416, 832 kg SO_4/ha) as seedlings fertilized with

NH_4NO_3+K_2SO_4 (98, 196, 393 kg SO_4/ha). Thus the increased photochemical efficiency may be due to an improved nitrogen:sulfur balance. Also, since chloride is required for the water splitting portion of the Hill reaction (Kelley and Izawa 1978), increased chloride levels may have affected the F_{var}/F_{max} as well.

An increase in seedling nitrogen content would be expected to affect the amount of fluorescence emitted by a seedling. Nitrogen is a component of chlorophyll a and b and the nitrogen increase may have led to a more efficient light harvesting capability which would be reflected in the higher fluorescence. The overall increase in fluorescence in seedlings fertilized with nitrogen over the unfertilized seedlings (Figure 21) may be the result of an increase in the nitrogen containing enzyme, Rubisco, which may lead to a more efficient reduction of CO_2. Increased photosynthesis was reported for seedlings containing intermediate levels of nitrogen (2.1%) than lower (1.2%) or higher (2.6%) levels (Hawkins et al. 1995). An increase in photosynthesis capability and efficiency may lead to an increase in stored carbohydrates.

The F_{var}/F_{max} pattern over time doesn't exhibit photoinhibition or cold acclimation seen with species of colder climates such as white spruce (Vidaver et al. 1991) and Scots pine. Due to the climate, coastal Douglas-fir is able to remain more photosynthetically active during the winter. However, it appears that by fertilizing with nitrogen, the seedlings became slightly more physiologically active than those not fertilized. The baseline fluorescence observed here is similar to the results of Fisker et al. (1995) for coastal Douglas-fir. Generally, the October 23 F_{var}/F_{max} values were the lowest and the December 9 values the highest. This may be because the summer drought (and the nursery imposed water stress for dormancy induction) had just ended by the first analysis date and the F_{var}/F_{max} reflected some water stress. Also, greater seedling root damage may have resulted during lifting and transplanting due to low soil moisture in the nursery.

3.5.3.2 Fluorescence of Freeze Treated Seedlings

Although F_{var}/F_{max} did not correlate well with seedling vitality in unfertilized seedlings, the F_{var}/F_{max} trend did parallel the declining seedling LT_{50} closely. These results differ from those of Fisker et al. (1995) where an initial decline in $LT_{50}F_{var}/F_{max}$ was found as Douglas-fir seedling LT_{50} initially declined. However, increases in $LT_{50}F_{var}/F_{max}$ were observed from early December to late January as seedling LT_{50} fell to - 20° C.

The declining $LT_{50}F_{var}/F_{max}$ is interesting in that there is not a static threshhold F_{var}/F_{max} level below which seedlings will consistently perish. In this instance, cold damage which resulted in low F_{var}/F_{max} observations did not necessarily result in seedling death. Although seedling vigor is no doubt negatively impacted, seedlings with low F_{var}/F_{max} are able to survive (as determined by visual assessment). Long term evaluation of the impact of freezing stress on seedlings using variable chlorophyll fluorescence would be interesting.

3.6 Conclusion

The null hypothesis of no treatment effect on seedling cold hardiness LT_{50} levels on each date was not rejected. The null hypothesis of no treatment effect on baseline chlorophyll fluorescence F_{var}/F_{max} levels was rejected. The hypothesis that chlorophyll fluorescence F_{var}/F_{max} of baseline freeze-treated seedlings does not correlate with seedling damage or mortality in unfertilized seedlings was not rejected.

Fall fertilization with nitrogen after dormancy has been induced did not negatively impact the acquisition of seedling cold hardiness in this study. Therefore, a seedling nutrition regime, which includes fall fertilization to nutrient load the seedlings prior to outplanting, may be a beneficial nursery cultural practice.

Increasing seedling nitrogen levels through fall fertilization led to significant increases in photochemical efficiency as measured by chlorophyll fluorescence. This may mean

additional carbohydrate reserves could be stored. However, further analysis into the relationship between photosynthetic rates and the seedling carbohydrate levels that result from increased nitrogen, and the increase in F_{var}/F_{max}, is necessary to refine the physiological significances of these findings.

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3.8 Appendix

3.8 Appendix

TABLE VI. P-values and mean squares for LT_{50}'s

Time	Effect	P value	MS
23-Oct	Block		
	Fert	LT50 could not be determined	
	Rate		
	FXR		
	MSE		
13-Nov	Block	0.0830	3.4679
	Fert	0.0592	5.2741
	Rate	0.0172	6.4994
	FXR	0.0002	16.9483
	MSE		1.5184
9-Dec	Block	0.0203	6.7090
	Fert	0.0385	6.8810
	Rate	0.4086	1.5999
	FXR	0.0184	6.4386
	MSE		1.5288
30-Dec	Block	0.9404	0.0653
	Fert	0.2934	0.5778
	Rate	0.5734	0.3386
	FXR	0.1115	1.1228
	MSE		0.4975

Chapter 4

4.0 Conclusion

Fall fertilization with nitrogen may be a beneficial practice for increasing seedling nitrogen levels. Within the scope of inference of this study, coastal Douglas-fir seedlings continue to take up and translocate nitrogen throughout the fall and winter. Both root and foliar TKN levels increased (interpreted as luxury consumption) under the conditions of this study. Applying potassium does not appear to stimulate luxury consumption if the seedlings already contain enough potassium. Applying chloride led to increased uptake of chloride, but there did not appear to be any detrimental effect on the seedlings. Other macro- and micronutrients not applied as fertilizers were generally not affected by the treatments and in most cases continued to increase during the course of the fall and winter. Due to the increases observed for TKN, nutrient ratios based off of TKN were significantly decreased. The biological significance is probably small, although the large difference created in the N/K ration may have some implications in the spring when active shoot growth resumes. Since K is needed more during this time of year, it is possible a deficiency K may have been created due the large increase in TKN, especially for the 160 and 320 kg N/ha treatments. The significant increase in budbreak timing is probably not biologically significant, given the obvious differences between the experimental (greenhouse) conditions and the natural environment.

Cold hardiness was not affected by the fertilizer applications. But given the amount of TKN increase (to 2.2%) and the mild winter, perhaps the results would have been different had there been a larger increase in foliar TKN or had there been some cold snaps. The increase observed in variable chlorophyll fluorescence F_{var}/F_{max} ratios suggests that the photosynthetic efficiency of fertilized seedlings is improved. It appears this enhancement is due largely to increases in foliar TKN. It is also interesting to note the differences between the types of fertilizer. Seedlings fertilized with chloride and with twice as much sulfate had consistently higher F_{var}/F_{max} ratios. This increase may mean the seedlings are

able to create larger starch reserves, but more research would be needed to help answer that question.

The deciding factor in determining whether to fall fertilize rests with the consensus of the nursery manager and reforestation specialist. If seedlings do not meet suggested foliar nitrogen levels by the end of the active shoot growth season then fall fertilization may be financially justified. Also, if a particular lot of seedlings are scheduled to be planted on a site known to be nutrient poor, elevating seedling nutrient reserves again may be warranted. Given similar circumstances and application methods, nursery managers should feel confident in their ability to increase seedling foliar nitrogen levels. More information is needed regarding the feasibility of increasing other nutrient levels. More information is also needed regarding how increased nitrogen nutrition affects outplanting performance. Until a better link is established, this nursery cultural practice will not be widely accepted.

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