THE EFFECTS OF NITROGEN RATE ON SEEDLINGS GROWTH IN PICEA MARIANA

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ABSTRACT

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Keywords: Nitrate concentration, the nitrogen cycle, photosynthetic efficiency, Picea Mariana, relative growth rate, root collar diameter

Nitrogen (N) is critically important for plant growth and development. The objective of this thesis is to examine how different rates of Nitrogen affect the seedling growth in black spruce (Picea mariana Mill.). This paper investigates the effects of nitrogen fertilization on seedlings growth for blcak spruce (Picea mariana Mill.) with a range of fertilizer applications from 10 to 360 µmol mol^{-1}N. The experiment was carried out in 2 environmentally controlled greenhouses in the Forest Ecology Complex at the Thunder Bay Campus of Lakehead University. After four months the fertilizer application, the seeding height, root collar diameter, and the height to diameter ratio were evaluated. Seedling growth was significantly affected by the rate of Nitrogen fertilizer, with an optimum rate at 150% nitrogen rate. The average heights were 38.31, 51, 46.01 43.93 and 47.81cm, respectively, for the 10, 150, 220, 290 and 360 umol mol^{-1} N treatments. The nitrogen treatments also affected the variability of RCD but the patterns of variation were different from that of seedling height. Both seedling heights and Root collar diameter decreased after 150 umol mol^{-1} N, which indicating that saturating plant with high nitrogen levels, however, does not improve plant growth.

CONTENT

FIGURES AND TABLES

ACKNOWLEDGMENTS

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INTRODUCTION

Nitrogen significantly affects eco-physiological traits as well as the growth and survival of plants. It is the most common nutrient element that limits forest productivity. It is also a common nutrient that greenhouse managers manipulate in order to control the rate of tree seedling growth and cold hardening. We must make sure that nitrogen supply is appropriate for tree seedlings during different phases of seedling development. Saturating plant with high nitrogen levels, however, does not improve plant growth. In fact, it can actually harm the trees. It is therefore critically important to investigate how tree seedlings respond to different rates of N fertilization.

Trees, like all living organisms which need various nutrients to grow and survive, obtain these elements from the soil. As conifers, spruce trees perform best with fertilizers that have nitrogen (N) levels higher than phosphate and potassium concentrations (Kay 2010). Of all of the elements necessary for tree growth the one nutrient that is most often in short supply is nitrogen (N). This is because nitrogen, in its elemental form (N2) is a gas, which makes it unavailable to most plants unless the plant can obtain it from the atmosphere (Kay 2010).

Most plants, like spruces, have to obtain the nitrogen they use from the soil or fertilizers in the form of either the nitrate (NO_3^-) or ammonium (NH_4^+) form. The nitrate form (NO_3^-) of nitrogen is highly soluble in water and will often leach out of the soil before plants have a chance to fully utilize all of it. This form is also somewhat unstable so that nitrogen reverts back to a gaseous state in the atmosphere in a process known as denitrification （Kay 2010）. Ammonium (NH_4^+), on the other hand, is more readily available to plants because it is more stable, a plant has to decompose organic matter before it can extract nitrogen through ammonium (Kay 2010).

To understand the effect of nitrogen fertilization on seedling growth, it is important to know the relative effect of nitrogen fertilization on growth of foliage and on the photosynthetic efficiency of the foliage. Previous studies have found that nitrogen significantly affects eco-physiological traits as well as the growth and survival of plants. Since N is the prime constituent of amino acids, growth regulators, and chlorophyll that drive the processes of photosynthesis and transpiration. These eco-physiological parameters rely heavily on N in order to function properly. Both photosynthesis and transpiration generally increase with increasing N input, but the effect will vary with species and stage of development (Tan and Hogan 1995, Kubiske et al. 1997). Increasing N application generally enhances height and diameter increments (Van den Driessche 1989, Catin et al. 1997), total leaf area (TLA, Sabate and Gracia 1994), foliar nitrogen concentration (Nf, Coleman et al. 1998), and photosynthetic water-use-efficiency (PWUE, Green and Mitchell 1992, Liu and Dickman 1996). On the other hand, increased N reduces photosynthetic nitrogen-use-efficiency (PNUE, Birk and Vitousek 1986, Kubiske et al. 1997) and biomass allocation to roots (R/S ratio, Fetene et al. 1993, Ibrahim et al. 1997). There are substantial differences between species in N requirement and allocation.

OBJECTIVE AND HYPOTHESIS

The objective of this study was to investigate eco-physiological responses to a range of N application rates (10 to 360 μmol $mol^{-1}N$) in black spruce (Picea Mariana (Mill.) B.S.P.). The main hypothesis was that increasing N soil availability would increase seedling height, root collar diameter growth. The N regime at 10 μmol $mol^{-1}N$ is the lowest level when the optimum concentrations for other essential nutrients are to be maintained. Except for the available soil N concentration reported for a black spruce forest (226 μmol $mol^{-1}N$) by Driscoll et al. (1999), others usually reported soil N in terms of mass over area, which is difficult to relate these values the application rates used in this study.

LITERATURE REVIEW

SOIL NITROGEN

Nitrogen is an essential nutrient for plant growth, development and reproduction. Despite nitrogen being one of the most abundant elements on earth, nitrogen deficiency is probably the most common nutritional problem affecting plants worldwide – nitrogen from the atmosphere and earth's crust is not directly available to plants (The Mosaic company 2016). Nitrates are most often used in commercial fertilizer because they are readily available to plants and can be used to quickly correct a severe deficiency (Kay 2016).

Of all the essential nutrients, nitrogen is required by plants in the largest quantity and is most frequently the limiting factor in crop productivity.

- In plant tissue, the nitrogen content ranges from 1 and 6%.
- Proper management of nitrogen is important because it is often the most limiting nutrient in crop production and easily lost from the soil system (University of Hawaii 2017).

Soil nitrogen exists in three general forms:

- organic nitrogen compounds;
- ammonium (NH^{4+}) ions;
- and nitrate (NO^{3-}) ions (The Mosaic company 2017).

The majority of plant-available nitrogen is in the inorganic forms NH^{4+} and NO^{3-}

(sometimes called mineral nitrogen). Ammonium ions bind to the soil's negatively charged cation exchange complex (CEC) and behave much like other cations in the soil. Nitrate ions do not bind to the soil solids because they carry negative charges, but exist dissolved in the soil water, or precipitated as soluble salts under dry conditions (The Mosaic company 2017).

Functions of nitrogen in plants:

- Nitrogen is an essential element of all amino acids. Amino acids are the building blocks of proteins;
- Nitrogen is also a component of nucleic acids, which form the DNA of all living things and holds the genetic code;
- Nitrogen is a component of chlorophyll, which is the site of carbohydrate formation (photosynthesis). Chlorophyll is also the substance that gives plants their green color;
- Photosynthesis occurs at high rates when there is sufficient nitrogen;
- A plant receiving sufficient nitrogen will typically exhibit vigorous plant growth. Leaves will also develop a dark green color (University of Hawaii 2016).

Natural Sources of Soil Nitrogen:

The nitrogen in soil that might eventually be used by plants has two sources: nitrogen-containing minerals and the vast storehouse of nitrogen in the atmosphere. The nitrogen in soil minerals is released as the mineral decomposes. This process is generally quite slow, and contributes only slightly to nitrogen nutrition on most soils. On soils containing large quantities of $NH4^{+-}$rich clays (either naturally occurring or developed by fixation of $NH4^{+}$ added as fertilizer), however, nitrogen supplied by the mineral fraction may be significant in some years (The Mosaic company 2017).

Atmospheric nitrogen is a major source of nitrogen in soils. In the atmosphere, it exists in the very inert N2 form and must be converted before it becomes useful in the soil. The quantity of nitrogen added to the soil in this manner is directly related to thunderstorm activity, but most areas probably receive no more than 20 lb nitrogen/acre per year from this source (The Mosaic company 2017).

Bacteria such as Rhizobia that infect (nodulate) the roots of, and receive much food energy from, legume plants can fix much more nitrogen per year (some well over 100 lb nitrogen/acre). When the quantity of nitrogen fixed by Rhizobia exceeds that needed by the microbes themselves, it is released for use by the host legume plant. This is why well-nodulated legumes do not often respond to additions of nitrogen fertilizer. They are already receiving enough from the bacteria (The Mosaic company 2017).

THE NITROGEN CYCLE

The heart of the nitrogen cycle is the conversion of inorganic to organic nitrogen, and vice versa. As microorganisms grow, they remove H4+ and NO3- from the soil's inorganic, available nitrogen pool, converting it to organic nitrogen in a process called immobilization. When these organisms die and are decomposed by others, excess NH4+ can be released back to the inorganic pool in a process called mineralization. Nitrogen can also be mineralized when microorganisms decompose a material containing more nitrogen than they can use at one time, materials such as legume residues or manures. Immobilization and mineralization are conducted by most microorganisms, and are most rapid when soils are warm and moist, but not saturated with water. The quantity of inorganic nitrogen available for crop use often depends on the amount of mineralization occurring and the balance between mineralization and immobilization (The Mosaic company 2017).

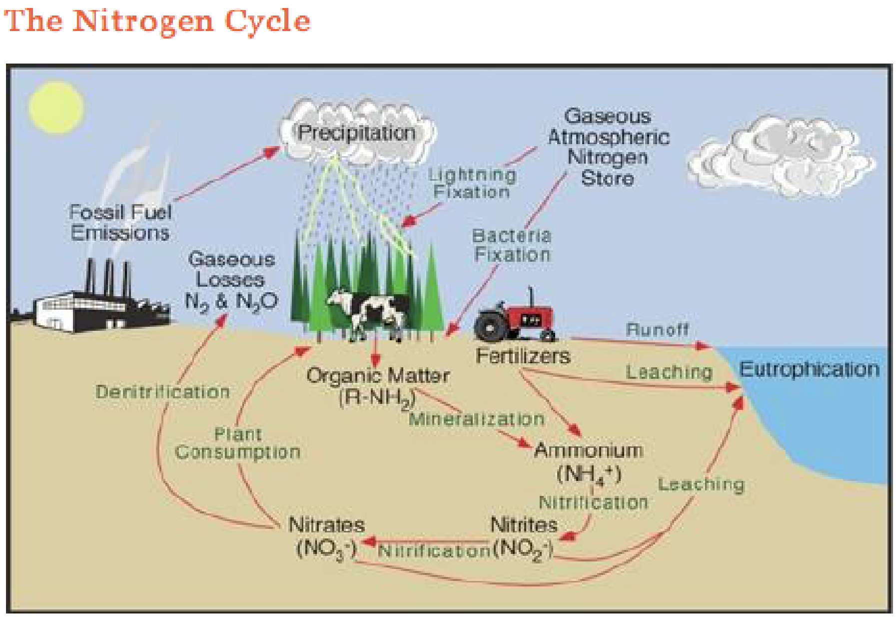


Figure1: The Nitrogen Cycle.
Source: http://www.physicalgeography.net

CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence is light re-emitted by chlorophyll molecules during return from excited to non-excited states and used as indicator of photosynthetic energy conversion in higher plants, algae and bacteria (Wikipedia 2016). Chlorophyll fluorescence emission has been proven a useful tool to determine photosynthetic activity (Vidaver et al. 1989). Chlorophyll fluorescence appears to measure of photosynthesis, but this is an over-simplification. Fluorescence can measure the efficiency of PSII photochemistry, which can be used to estimate the rate of linear electron transport by multiplying by the light intensity. However, researchers generally mean carbon fixation when they refer to photosynthesis. Electron transport and CO2 fixation can correlate well, but may not correlate in the field due to processes such as photorespiration, nitrogen metabolism and the Mehler reaction (Wikipedia 2016). Fluorescence assessment has been used to provide information about the physiological status of white spruce (Vidaver et al. 1989). Generally, the chlorophyll fluorescence emission is an indicator of Photosystem II activity, quantifying the quantum yield, in isolated photosynthetic membranes (Lambers et al. 1998). It is normally referred to as photosystem II, since it originates mainly from Photosystem II (Lambers et al. 1998). For the purpose of this thesis, the quantum efficiency of photosystem II is referred to as Photosystem II. Values for Photosystem II vary based on the degree of illumination to which the samples are exposed. Under dark-incubated conditions, the values for Photosystem II are around 0.8 (relative units) in healthy leaves (Lambers et al. 1998). Under illuminated conditions Photosystem II has values equal to or lower than that of dark-incubated samples, and the difference increases with increasing irradiance (Lambers et al. 1998).

PHOTOSYNTHESIS AND NITROGEN-USE EFFICIENCY

In C3 crop plants about 60–80% of leaf nitrogen (N) is invested in the photosynthetic apparatus, and N nutrition plays a crucial role in determining photosynthetic capacity (Kumar et al. 2002). The proportion of leaf N invested in photosynthetic components is fairly constant. By contrast, both N per unit leaf area and the allocation of N between the component photosynthetic processes depend on environmental factors such as N availability, irradiance and CO2 concentration (Kumar et al. 2002). Light-harvesting and electron transport components often show a co-ordinated and equivalent response to N nutrition (Kumar et al. 2002). In contrast, most studies have shown disproportionately large changes in ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in response to N supply, demonstrating the importance of this protein in leaf N economy. At low light, for a given N availability, more protein is allocated towards light harvesting components in order to maximize light capture and, expressed per unit Chl, electron transport and carboxylation capacities are relatively small. High irradiance tends to alter the partitioning of N away from thylakoid protein to soluble proteins, particularly Rubisco. Growth at elevated CO2 often leads to decreases in the amounts of Rubisco and other photosynthetic components on a leaf area basis. This is explicable in terms of greater N sinks elsewhere in the plant as a result of increased carbohydrate availability and acclimatory changes

(Kumar et al. 2002). Models predict that in order to arrive at optimal N use efficiency (NUE) at likely future ambient CO2 concentrations, leaves will need to achieve a redistribution of N so that the ratio between the capacities for regeneration of ribulose-1,5-bisphosphate and carboxylation increases by 30–40%. Human intervention to improve the NUE of crops would have economic and environmental benefits, reducing pollution of water supply by nitrates. The NUE of photosynthesis could be increased either through manipulation of Rubisco amounts or properties, or by decreasing photorespiration. While decreasing Rubisco content could enhance NUE by only about 5%, eliminating photorespiration could produce a change of more than 50% (Kumar et al. 2002).

NITROGEN USE EFFICIENCY

Nitrogen Use Efficiency (NUE) is a term used to indicate the ratio between the amount of fertilizer N removed from the field by the crop and the amount of fertilizer N applied (Brentrup &Palliere 2010).

- can be calculated as the ratio between the amount of fertilizer N removed with the crop and the amount of fertilizer N applied;
- expressed in %;
- provides information about the relative utilization of additional N applied to an agricultural production system of a country or region;
- NUE considers productivity more than the N balance (Brentrup &Palliere 2010).

MATERIALS AND METHODS

PLANT MATERIALS:

One-year-old black spruce (Picea mariana [Mill.] B.S.P.) seedlings were obtained from Hill's Tree Seedling Nursery in Thunder Bay, Ontario. The seedlings were relatively uniform in size at the beginning of the experiment (H=22.8±0.16cm, RCD=2.05±0.02mm). All seedlings were potted in 165-styroblock containers and filled up with a mixture of vermiculite and peat moss (50:50).

EXPERIMENTAL DESIGN AND NITROGEN TREATMENT

The experiment was carried out in two environmentally controlled greenhouses in the Forest Ecology Complex at the Thunder Bay Campus of Lakehead University. The treatment consisted six nitrogen concentrations: 10, 80, 150, 220, 290 and 360 μmol mol^{-1}N. The N-P-K ratios were 5/2/5 in all N treatments. These were optimal ratios for conifer seedlings (Landis, 1993). One tenth of the optimal N supply concentration (10 μmol mol^{-1}N) is considered a very low N supply level (Zhang et al. 2006). The treatments were replicated in each of the greenhouses with six seedlings in each N –greenhouse combination and a total of 72 seedlings. The location of each nitrogen treatment within each greenhouse was randomized. The day/night air temperatures were controlled at 25-26/16-17 ^{0}C and the photoperiod was set at 16 hours (natural daylength was extended using high-pressure sodium lamps when natural daylength is less than 16 hours) in both greenhouses. All the experiment conditions (temperature, RH, [CO_2] and light) were monitored and controlled using a computerized Argus control system (Argus Control Systems Ltd, Vancouver, BC, Canada). All the seedlings were fertilized three time a week to maintain water content above 30% (by volume) (Bergeron *et al.* 2004) in the growing medium. The experiment lasted 4 months. The height and root collar diameter of the tree seedlings were measured, and the height to diameter ratio was calculated and used as one of the seedling quality indicators.

MEASURED VARIABLES

Height and root collar diameter and the height to diameter ratio was calculated on each seedling. The measurements were taken after 120 days of treatments.

Seedling height was measured in centimeters (cm) using a 5m Mark Truper measuring tape, FH-3m (Truper, Taiwan), with an accuracy of 0.01mm, that was placed vertically on the substrate surface. The root collar diameter was measured using a with a 150mm LCD Electronic Digital Vernier calliper (Grainger, USA). The unit of measurement was millimeters (mm). The root collar diameter was measured on the main stem of the plant 1cm above the substrate. The measurements for each trial can be found in Appendix 1.

DATE ANALYSIS

The data were subjected to analysis of variance using the General Linear Model for a Randomized Complete Block Design to obtain the P value of the effect of the model for each treatment. For the statistical analysis, I used two-way analysis of variance (ANOVA) and multiple comparisons (Least Squares Difference (LSD) Post Hoc) for each variable and treatment. The level of statistical significance was set at (p< 0.05) for all the analyses within a confidence interval of 95%.

RESULTS

Seedling Height

Table 1. Seedling height results from 2-way ANOVA

Tests of Between-Subjects Effects

Dependent Variable: Height

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1625.889[a]	11	147.808	3.885	.000
Intercept	147659.894	1	147659.894	3880.788	.000
NitrogenLevel	1094.438	5	218.888	5.753	.000
GreenHouse	267.576	1	267.576	7.032	.010
NitrogenLevel * GreenHouse	263.876	5	52.775	1.387	.242
Error	2282.937	60	38.049		
Total	151568.720	72			
Corrected Total	3908.826	71			

a. R Squared = .416 (Adjusted R Squared = .309)

Both nitrogen supply and greenhouse has significant effects on seedling height (Table 1). The Least Squares Difference (LSD) Post Hoc test shows that the 150, 220 and 360 umol mol^{-1} N levels resulted in significantly greater seedling height growth than the 10 umol mol^{-1} N treatment (Figure 2). The average heights the were 38.31, 51, 46.01 43.93 and 47.81cm respectively with the treatment of 10, 150, 220, 290 and 360 umol mol^{-1} N (Figure 2).

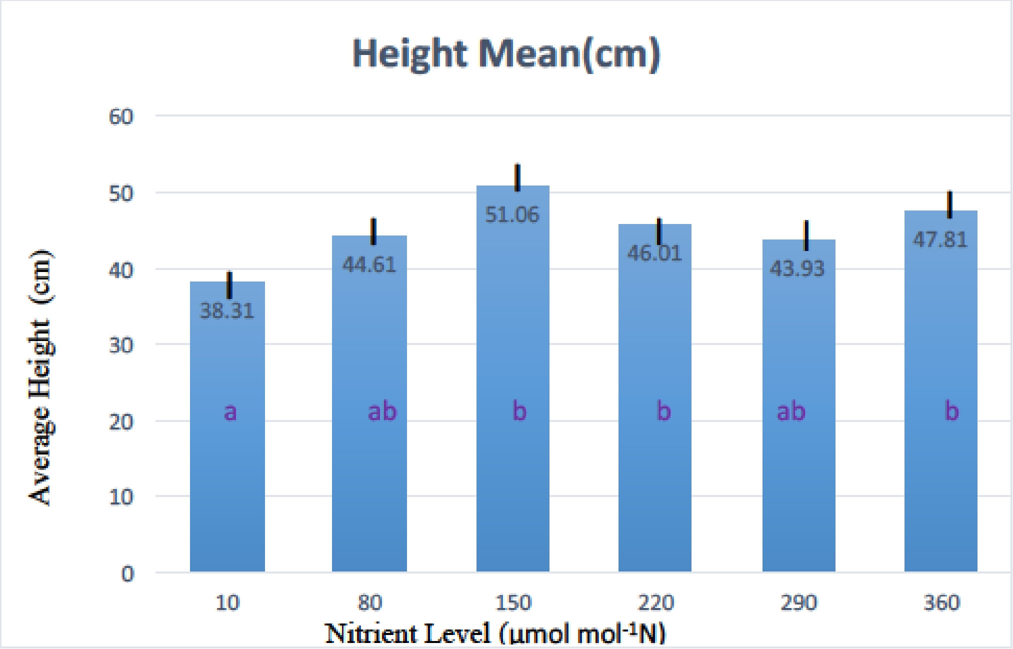


Figure 2: Average seedling height (cm) of black spruce for the 6 treatments with LSD significance.

Letters were assigned to the data to indicate whether there was any significant difference between the data. Common letters indicate no significance while different letters indicate that there is statistical significance.

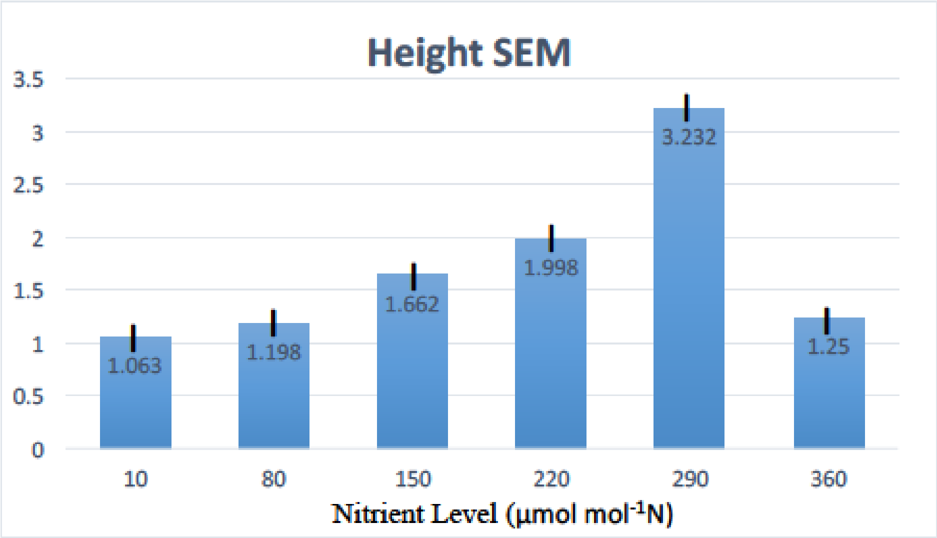


Figure 3: Height of black spruce SEM for the 6 treatments.

The application of N influenced plant height variability (Figure 3). The variability was the highest in the 290 nitrogen level and became smaller as the application rate deviated from that level in either direction.

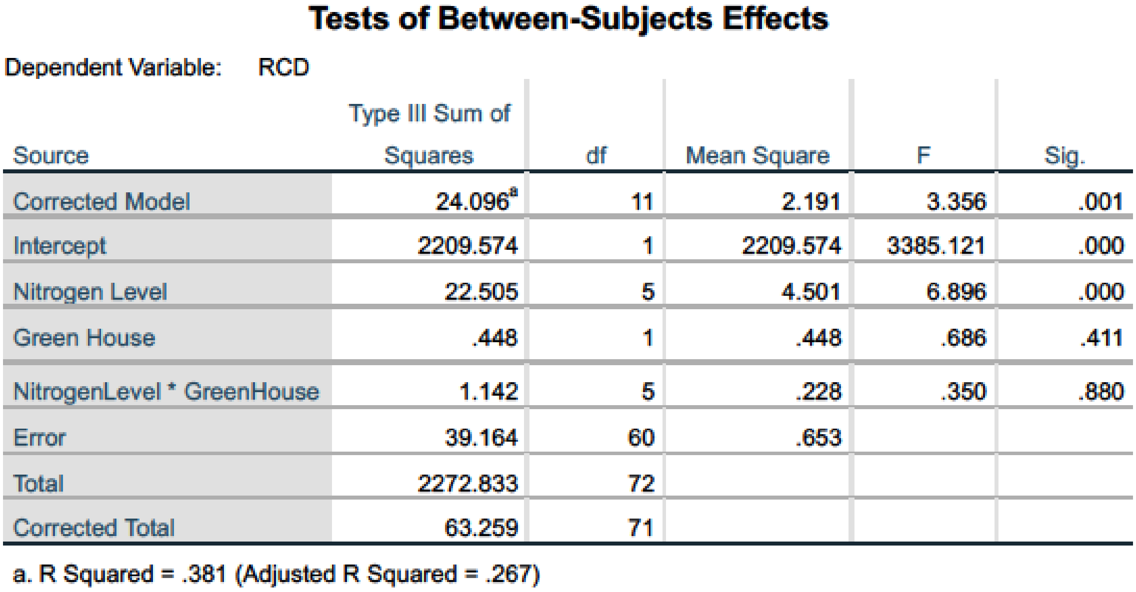
Table 2: Multiple Comparisons of Means of Height.

Multiple Comparisons of Means				
Linear Hypotheses:				
Estimate Std.Error			t value	Pr(>|t|)
Level10-80	6.3000	2.5554	2.4650	0.1500
Level10-150	12.7500	2.5554	4.9890	< 0.001 ***
Level10-220	7.7000	2.5554	3.0130	0.04102 *
Level10-290	5.6167	2.5554	2.1980	0.2528
Level10-360	9.5000	2.5554	3.7180	0.00548 **
Level80-150	6.4500	2.5554	2.5240	0.1323
level80-220	1.4000	2.5554	0.5480	0.9939
level80-290	-0.6833	2.5554	-0.2670	0.9998
level80-360	3.2000	2.5554	1.2520	0.8093
level150-220	-5.0500	2.5554	-1.9760	0.3670
level150-290	-7.1333	2.5554	-2.7910	0.0716
level150-360	-3.2500	2.5554	-1.2720	0.7991
level220-290	2.0833	2.5554	-0.8150	0.9637
level220-360	1.8000	2.5554	0.7040	0.9808
level290-360	3.8833	2.5554	1.5200	0.6531

Seedling height was significantly different between the following treatments: 10 versus 150 (P= 0.001), 10 versus 220 (P= 0.04102) and 10 versus 360 (P = 0.00548).

Root Collar Diameter:

Table 3: RCD results from 2-way ANOVA.



Tests of Between-Subjects Effects

Dependent Variable: RCD

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24.096[a]	11	2.191	3.356	.001
Intercept	2209.574	1	2209.574	3385.121	.000
Nitrogen Level	22.505	5	4.501	6.896	.000
Green House	.448	1	.448	.686	.411
NitrogenLevel * GreenHouse	1.142	5	.228	.350	.880
Error	39.164	60	.653		
Total	2272.833	72			
Corrected Total	63.259	71			

a. R Squared = .381 (Adjusted R Squared = .267)

Nitrogen treatment significantly affected the root collar diameter but greenhouse did not (Table 3). The probability value for nutrient treatment was 0.000, much smaller than the 0.05 threshold. The probability for Greenhouse was 0.395, indicating that it is not significant. Root collar diameter of seedlings generally increased with increase in fertilizer rate until level 150. However, it was observed that the mean stem base diameter from 150 and 290 µmol mol^{-1}N levels were significantly higher and identical followed by plants subjected to 360 and 220 µmol mol^{-1}N which was statistically greater than those treated with 10 µmol mol^{-1}N. In level 10, average RCD for black spruce was 4.62 mm, significantly smaller than the 6.23 mm for level 150, 5.65 mm for level 220 and 5.83mm for level 360 (Table 4, Figure 4). The nitrogen treatments also affected the variability of RCD but the patterns of variation were different from that of seedling height (figures 3 and 5).

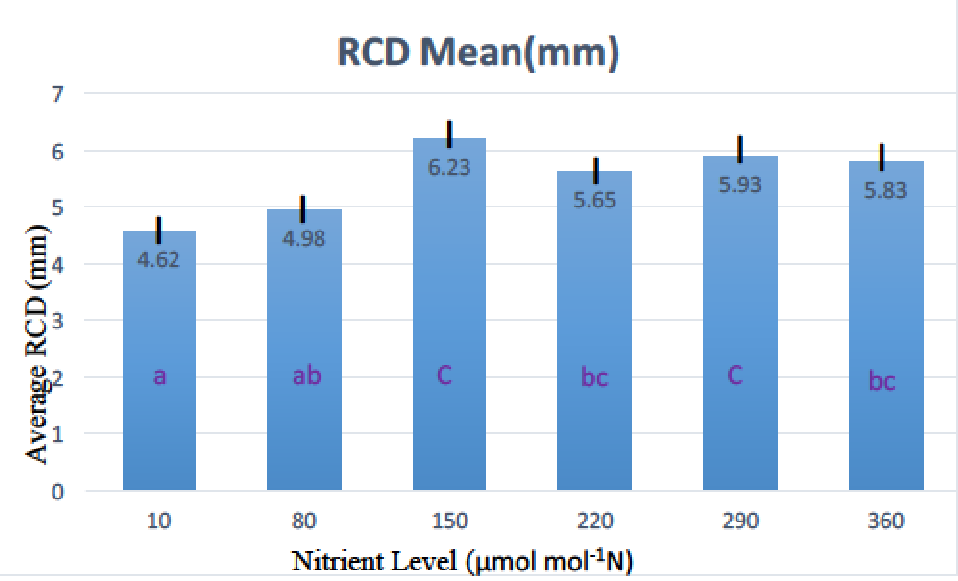


Figure 4: Average RCD (mm) of black spruce for the 6 treatments with LSD significance.

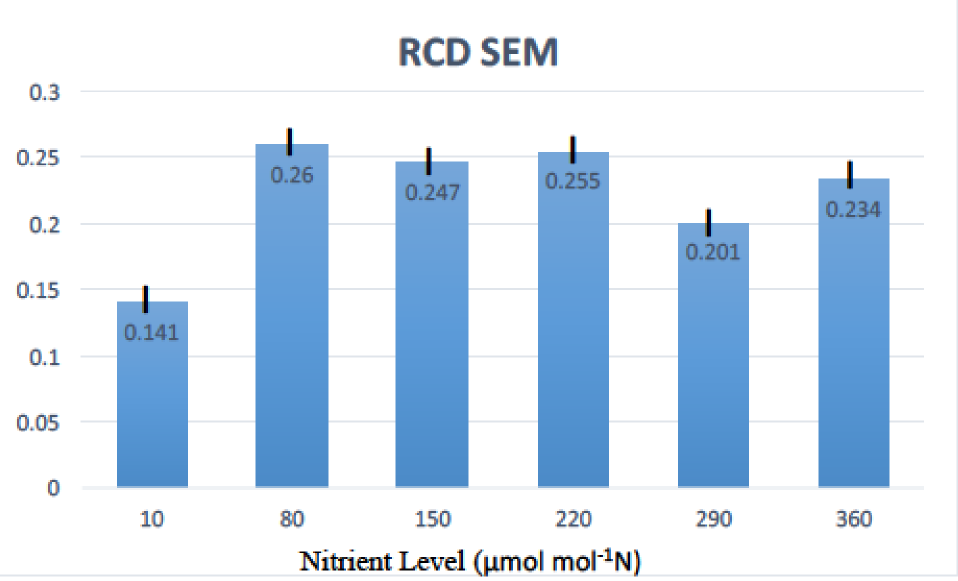


Figure 5: RCD of black spruce SEM for the 6 treatments.

The application of N influenced plant RCD variability (Figure 5). The variability was the highest in the 80 nitrogen level and followed by 220 and 150 nitrogen level.

Table 4: Multiple Comparisons of Means of RCD.

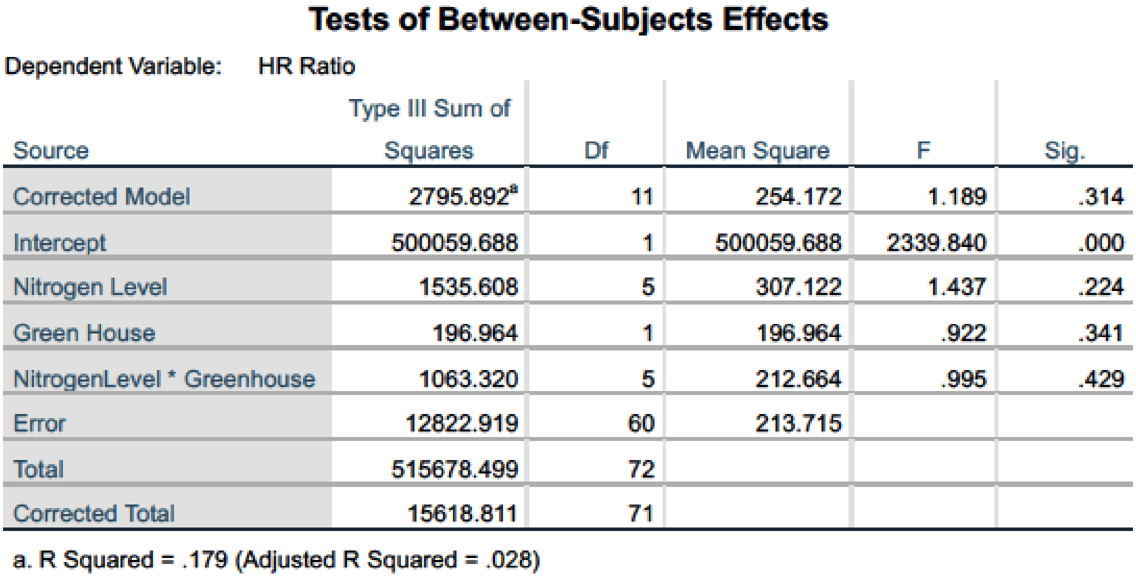
Multiple Comparisons of Means				
Linear Hypotheses:				
Estimate Std.Error			t value	Pr(>|t|)
Level10-80	0.3542	0.3215	1.1020	0.8789
Level10-150	1.6042	0.3215	4.9900	< 0.001 ***
Level10-220	1.0225	0.3215	3.1810	0.02630 *
Level10-290	1.3025	0.3215	4.0520	0.00183 **
Level10-360	1.2100	0.3215	3.7640	0.00464 **
Level80-150	1.2500	0.3215	3.8880	0.00314 **
level80-220	0.6683	0.3215	2.0790	0.3113
level80-290	0.9483	0.3215	2.9500	0.04818 *
level80-360	0.8558	0.3215	2.6620	0.0972
level150-220	-0.5817	0.3215	-1.8090	0.4671
level150-290	-0.3017	0.3215	-0.9380	0.9350
level150-360	-0.3942	0.3215	-1.2260	0.8225
level220-290	0.2800	0.3215	0.8710	0.9521
level220-360	0.1875	0.3215	0.5830	0.9918
level290-360	-0.0925	0.3215	-0.2880	0.9997

The seedling RCD in the 10 μmol mol^{-1} N treatment was significantly smaller than all other treatment levels (Table 4, Figure 4). The 80 and 290 μmol mol^{-1}N treatments also resulted in significantly larger RCD than the 80 μmol mol^{-1}N (Table 4, Figure 4).

H/R Ratio:

The height to diameter ratio in black spruce seedlings was not significantly affected by any of the treatments (Table 4).

Table 4: H/R results from 2-way ANOVA.



Tests of Between-Subjects Effects

Dependent Variable: HR Ratio

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2795.892[a]	11	254.172	1.189	.314
Intercept	500059.688	1	500059.688	2339.840	.000
Nitrogen Level	1535.608	5	307.122	1.437	.224
Green House	196.964	1	196.964	.922	.341
NitrogenLevel * Greenhouse	1063.320	5	212.664	.995	.429
Error	12822.919	60	213.715		
Total	515678.499	72			
Corrected Total	15618.811	71			

a. R Squared = .179 (Adjusted R Squared = .028)

DISCUSSION

Greater seedling growth was observed with nitrogen treatment 150 µmol mol^{-1}N and slower growth, as expected, was in the 10 µmol mol^{-1}N treatment. Treatment 150 µmol mol^{-1}N, therefore, proved to be the most suitable nitrogen level. The seedling height showed that treatments with concentrations 10-150, 10-220 and 10-360 were significantly different in seedling growth. In addition, the height of seedling does not increase when nitrogen content exceeds 220 µmol mol^{-1}N.

The Root Collar Diameter results indicate the quality of the black spruce seedling.

A thicker Root Collar Diameter represents a lower probability that the tree will bend after being planted in the field and will be more competitive. The results of this study suggest that the Root Collar Diameter gradually increases when the concentration of nitrogen increases. However, there was no further improvement in stem diameter for nitrogen application rates beyond 150 μmol mol^{-1}N: 6.23mm, again suggesting this is probably the best application rate for growing black spruce seedlings among all the rates investigated in this study.

Generally, increases in N supply enhanced Seedling heights and Root Collar Diameter. At high N supply, particularly at 220 μmol mol^{-1}N, the height and Root Collar Diameter of black spruce seedlings did not increase, probably because of toxic N conditions. Toxic N conditions were evidenced by the substantial physiological and growth reductions meanwhile foliar N concentration continued to increase (Van den Driessche 1991). This was also evident in our study, especially in treatments beyond 175 ppm N (Figures 1 and 2). Conditions of luxury consumption of N can be detected when plant N concentration continues to increase whereas growth is stable (Van den Driessche 1991), and the luxury consumption range is intermediate between optimum and toxic N supply.

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APPENDICES

APPENDIX I: Seedling Measurement Data

Green House	Nitrogen Level	RCD	Height	HR Ratio
1	10	4.84	36.6	75.62
1	10	4.18	33.2	79.43
1	10	4.25	37.8	88.94
1	10	4.27	42.5	99.53
1	10	5.35	45.4	84.86
1	10	5.24	37.5	71.56
1	80	5.86	44.4	75.77
1	80	4.31	41.6	96.52
1	80	3.52	41.7	118.47
1	80	5.87	47.6	81.09
1	80	5.22	39.5	75.67
1	80	4.93	40.4	81.95
1	150	4.84	47.3	97.73
1	150	7.34	48.2	65.67
1	150	5.29	49.6	93.76
1	150	5.03	39.8	79.13
1	150	7.14	46.7	65.41
1	150	6.46	59.7	92.41
1	220	4.03	43.4	107.69
1	220	6.41	47.2	73.63
1	220	4.23	36.0	85.11
1	220	5.63	47.0	83.48
1	220	6.95	44.5	64.03
1	220	6.81	30.2	44.35
1	290	5.86	57.6	98.29
1	290	4.84	40.6	83.88
1	290	4.71	42.2	89.60
1	290	7.06	17.8	25.21
1	290	6.21	43.6	70.21
1	290	6.60	41.2	62.42
1	360	5.54	49.4	89.17
1	360	5.16	51.5	99.81
1	360	4.88	44.3	90.78
1	360	6.65	50.5	75.94
1	360	6.61	52.0	78.67

1	360	4.47	42.4	94.85
2	10	3.94	37.6	95.43
2	10	4.76	37.9	79.62
2	10	3.94	37.1	94.16
2	10	4.87	38.0	78.03
2	10	4.83	33.1	68.53
2	10	5.02	43.0	85.66
2	80	5.11	47.7	93.35
2	80	3.70	38.6	104.32
2	80	6.47	47.0	72.64
2	80	4.89	51.0	104.29
2	80	4.30	46.3	107.67
2	80	5.56	49.5	89.03
2	150	6.54	59.2	90.52
2	150	6.72	52.8	78.57
2	150	6.69	54.9	82.06
2	150	6.92	56.2	81.21
2	150	6.35	50.1	78.90
2	150	5.42	48.2	88.93
2	220	5.64	52.3	92.73
2	220	5.42	49.7	91.70
2	220	5.76	53.6	93.06
2	220	6.05	52.5	86.78
2	220	5.38	46.2	85.87
2	220	5.45	49.5	90.83
2	290	6.46	53.7	83.13
2	290	5.66	49.0	86.57
2	290	6.05	37.4	61.82
2	290	5.82	40.9	70.27
2	290	6.41	41.6	64.90
2	290	5.44	61.5	113.05
2	360	6.07	47.6	78.42
2	360	5.98	41.2	68.90
2	360	6.25	44.5	71.20
2	360	6.09	54.2	89.00
2	360	5.12	44.3	86.52
2	360	7.19	51.8	72.04