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Effects of environmental factors on seed germination

By:
Manchang Liang

A Thesis Submitted in Partial Fulfillment of the Requirements for
the Degree of Bachelor of Science in Forestry

FACULTY OF NATURAL RESOURCES MANAGEMENT
LAKEHEAD UNIVERSITY
THUNDER BAY, ONTARIO

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Abstract

Keywords: tree seed germination, environmental factors, light, planting depth

Detailed understanding of the environmental conditions required for the germination and growth of tree seeds is an important prerequisite for the formulation of comprehensive and sustainable forest development strategies, which is of great significance for afforestation and forest management. The germination and establishment of seeds under different light intensity, different planting depth and different species types were studied. Among all tested tree seeds, there was no significant difference in seed germination rates in different planting depths. There was no significant three-way interaction between light intensity, planting depth and tree species. There was a significant two-way interaction between light intensity and tree species. Under the same light intensity, the germination rate of black spruce was higher than that of Jack pine. Light, had significant effects on germination rate. After comparing between groups, it was found that the germination rate was higher under 60% light intensity.

Introduction

Jack pine (*Pinus banksiana* Lamb.) is the most widely distributed pine species in Canada (Rudolph and Laidly, 1990) and has significant economic importance to the forest industry (Foster and Morrison, 1976; Anonymous, 1980; Rudolph and Laidly, 1990). Jack pine can grow in almost any soil, but it needs full sun light and prefers dry soil although it can tolerate a range of moisture levels.

Jack pine is known for its unique shape and ability to thrive in the poorest conditions. Jack pine is a native species that has been used as food and medicine by the first nations for centuries. It almost grows in all province in Canada. Jack pine also plays an important role in reforestation after forest fires. The fire caused the cones to open and release the seeds, so that seedlings will regenerate at the first time after fire.

Recent years, studies in Ontario and whole Canada are attempting to solve the issues related to forest sustainable and the impacts of existing forest management practice on forest sustainable and biodiversity. Jack pine ecosystem productivity project is one of several related studies in North America.

Black spruce (*Picea mariana* (B.S.P.) Mill.) is the most common boreal tree species, and it is also called a transcontinental species. Black spruce is widely distributed in Canada, and it can be found across Ontario. Black Spruce is an adaptive species and they can grow in a variety of soils. For the shade tolerate, Black Spruce only can tolerate partial shade.

The tree is used in industry and commerce primarily for pulp and paper due to its unique properties - long fibers, but it is also used in the production of other wood products. Other indigenous people use it to make snowshoe stands and clotheslines.

Black spruce is also considered as a serotinous species, so that it also plays the same role with Jack pine in reforestation. That means it's still fire help open its cones and

release seeds. For example, Manitoba didn't have that many kinds of trees to begin with, only about 24. Black spruce is one of the most important tree species in most of the central and northern Manitoba province and thus plays an important role in creating forest habitats for many other plant and animal species. Black spruce provides food and shelter for the animals throughout the northern forest.

Germination of seeds is the initial, and under some circumstances, a critical step in the reproduction of forest stand by natural and artificial means. A fundamental knowledge of the ecology of germination of the seed of the commercially important species is therefore essential to the forester practicing the art of silviculture. (Ackerman 1965). Germination also is one of the most important factors to assess the quality of seeds depending upon species. These requirements are dictated by the species "tolerance" characteristics and the amount of stored substrate in the seed (Farmer 2017).

Jack pine seeds germinate best in mineral soils or organic. Some shade may help germinate, but young jack pines need plenty of sunlight to grow. In well-watered forest conditions, seeds germinate when the temperature reaches 18° C, but light also affects germination. Under continuous light conditions, germination is completed in the temperature range of 16 °~ 27° C (60 °~ 80° F).

Black Spruce seeds germinate and grow relatively easily. Seed dormancy is short, easily broken, and sowing without any pretreatment can be reasonably successful. The advantage of short pretreatment is that seeds germinate at a greater rate, and the germination of the seedlings will be synchronized with that of most seedlings within a few days.

It is now firmly established that after germination, tree seedlings have highly variable light requirements. In this study, the objective was to evaluate the relationship between light and germination rate, depth and germination rate, or both and germination rate. At

the same time, the study also will compare the difference between jack pine and black spruce. This study was under controlled conditions and use quantitative methods to evaluate the results.

Materials and methods

Materials

This study was carried out in greenhouse, at the Faculty of Natural Resources Management, Lakehead University, Canada. Jack pine seeds and white spruce seed was collected from the forest in Thunder Bay and store at minus temperature before using.

Experiment Design

The experiment was designed to study the relationship between germination and light plus depth for each species. So, there are two factors. The experiment design was two factor factorials (4 x 4) arranged in four designed units. This experiment was designed to study combined effects of light and depth factors.

Each species was designed as four units. Each unit has different light treatment, they are 20%, 60%, 80% and 100% light treatments. Each unit has four groups with different depth treatment, they are 0cm, 1cm, 2cm, 3cm. Each group has four replications and 9 seeds per replicate.

For each species, each pot was filled with soil first. And then, press the soil to the same level, to make sure each pot has the same soil. Second, use a stick with a length mark to dig holes. Each pot was dug three holes, and the three holes form an equilateral triangle at the center of each pot. The unit which don't have depth treatment also was chose three points form an equilateral triangle at the center of the pot. Third, put three seeds in the holes (or points), fill the holes in each pot with the soil we dug. The pots in each unit are mixed and placed. The light treatment uses shade cloth to control. One unit

doesn't use it, one unit uses 20% shade cloth, one unit uses 40% shade cloth and one unit uses double 40% shade cloth. Put the pots in the same place and take care of them every day to record data.

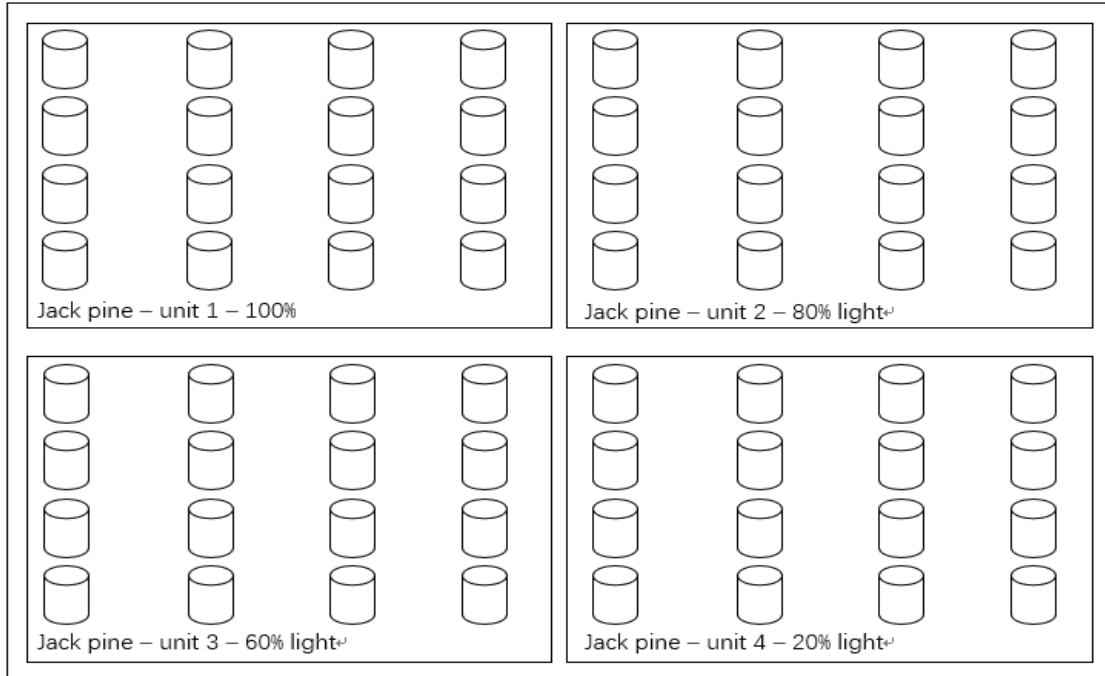


Figure 1: experiment design for jack pine

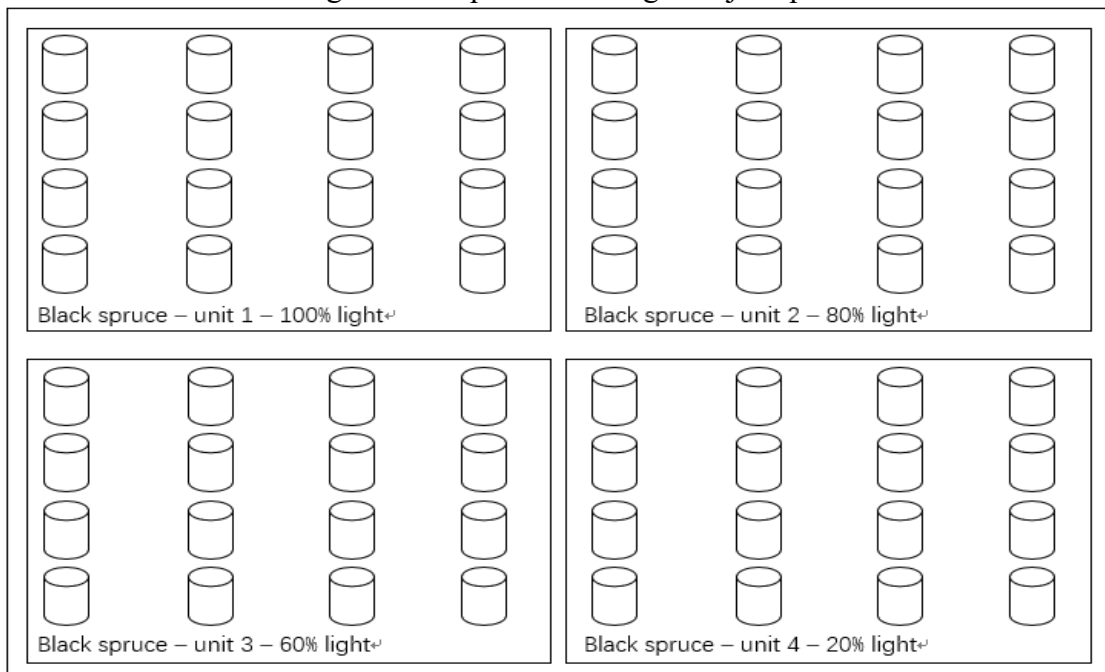


Figure 2: experiment design for Black spruce

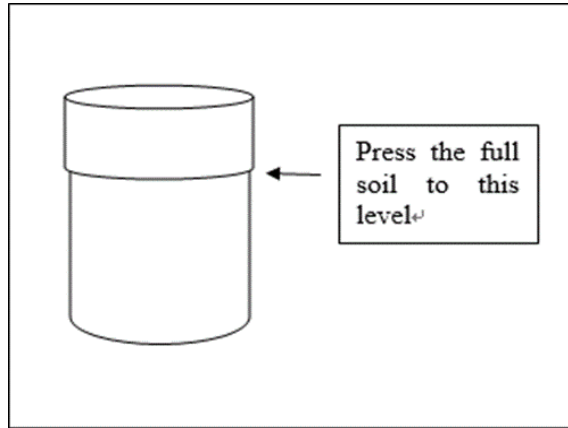


Figure 3: the level of soil

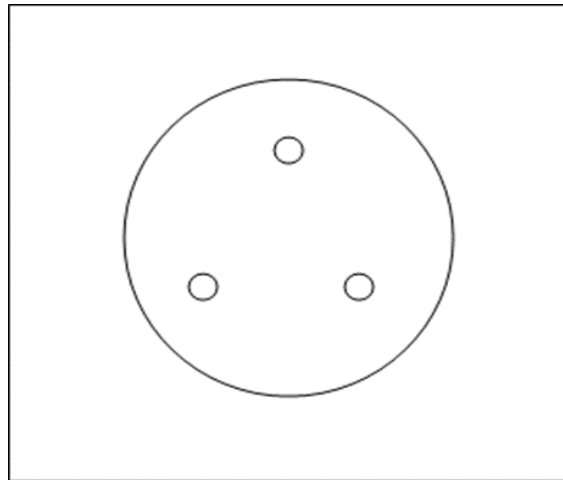


Figure 4: The holes in each pot

Statistical analysis

In whole process of the experiment, data were analyzed by analysis of variance followed by Tukey's Honestly Significant Difference test to compare the significant effects among species, light and depth. Significant differences among treatments were considered at the $P < 0.05$ level.

In the statistical analysis process, we analyzed according to the following ideas.

First, the analysis is three-factor ANOVA, Three-factor ANOVA is very sensitive to outliers. The analysis of the presence or absence of significant outliers was first performed.

In this study, data normality was tested by Shapiro-Wilk test. The analysis of whether the data conform to the normal distribution is carried out in the second step.

Levene's Test of Equality of Error Variances were used to verify whether the dependent variables in any classification of independent variables have equal variance. This is the third step.

The fourth step is to determine whether there is a significant three-factor interaction. If there is a three-factor interaction, then judge whether there is a simple two-factor interaction. If there is no three-factor interaction, then judge whether there is a two-factor interaction.

The next step is to judge the possible situation. If there is a simple interaction between two factors, judge whether there is a simple single factor effect; If there is no simple interaction between the two factors, the analysis is concluded.

The last step is a follow-up to the previous step. If there is a simple factor effect, the simple pairwise comparison is meaningful. If there is no effect, end the analysis.

Result and discussion

Analysis of significant outliers



Figure 5: analysis of significant outliers for Jack pine



Figure 6: analysis of significant outliers for black spruce

From the test results, the figure 5 and 6 show that there are no significant outliers.

Shapiro-Wilk test

Tests of Normality									
Species	Depth	Light		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
				Statistic	df	Sig.	Statistic	df	Sig.
Pj	0	20.00%	Germination rate	.151	4	.	.993	4	.972
		40.00%	Germination rate	.260	4	.	.827	4	.161
		60.00%	Germination rate	.441	4	.	.630	4	.001
		80.00%	Germination rate	.260	4	.	.827	4	.161
	1	20.00%	Germination rate	.151	4	.	.993	4	.972
		40.00%	Germination rate	.307	4	.	.729	4	.024
		60.00%	Germination rate	.290	4	.	.863	4	.271
		80.00%	Germination rate	.394	4	.	.773	4	.062
	2	20.00%	Germination rate	.298	4	.	.849	4	.224
		40.00%	Germination rate	.302	4	.	.827	4	.161
		60.00%	Germination rate	.298	4	.	.849	4	.224
		80.00%	Germination rate	.151	4	.	.993	4	.972
	3	20.00%	Germination rate	.329	4	.	.895	4	.406
		40.00%	Germination rate	.236	4	.	.911	4	.488
		60.00%	Germination rate	.329	4	.	.895	4	.406
		80.00%	Germination rate	.303	4	.	.791	4	.086
Sb	0	20.00%	Germination rate	.283	4	.	.863	4	.272
		40.00%	Germination rate	.307	4	.	.729	4	.024
		60.00%	Germination rate	.441	4	.	.630	4	.001
		80.00%	Germination rate	.441	4	.	.630	4	.001
	1	20.00%	Germination rate	.283	4	.	.863	4	.272
		40.00%	Germination rate	.283	4	.	.863	4	.272
		60.00%	Germination rate	.298	4	.	.849	4	.224
		80.00%	Germination rate	.298	4	.	.849	4	.224
	2	20.00%	Germination rate	.208	4	.	.950	4	.714
		40.00%	Germination rate	.302	4	.	.827	4	.161
		60.00%	Germination rate	.283	4	.	.863	4	.272
		80.00%	Germination rate	.260	4	.	.827	4	.161
	3	20.00%	Germination rate	.441	4	.	.630	4	.001
		40.00%	Germination rate	.364	4	.	.840	4	.195
		60.00%	Germination rate	.	4	.	.	4	.
		80.00%	Germination rate	.283	4	.	.863	4	.272

a. Lilliefors Significance Correction

Figure 7: Test of Normality

In this study, data normality was tested by Shapiro-Wilk test. In general, if the p-value of Shapiro-Wilk test is less than 0.05, we consider the data not in line with the normal distribution. The Numbers marked red in the table are displayed that a total of 5 sets of data did not conform to the normal distribution. They are Jack pine group with

a planting depth of 0cm and a light intensity of 60%; Black spruce group with planting depth of 0cm and illumination intensity of 60% and 80%; The black spruce group was planted at a depth of 3cm with a light intensity of 20% and 60%.

Levene's Test of Equality of Error Variances

Levene's Test of Equality of Error Variances were used to verify whether the dependent variables in any classification of independent variables have equal variance.

		Levene Statistic	df1	df2	Sig.
Germination rate	Based on Mean	2.975	31	96	.000
	Based on Median	1.580	31	96	.048
	Based on Median and with adjusted df	1.580	31	31.154	.104
	Based on trimmed mean	2.778	31	96	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
 a. Dependent variable: Germination rate
 b. Design: Intercept + Species + Depth + Light + Species * Depth + Species * Light + Depth * Light + Species * Depth * Light

Figure 8: Levene's Test of Equality of Error Variances

In general, if the P value of Levene variance homogeneity test is greater than 0.05, we consider the data conform to the equal variance. As can be seen from the above table, the P value of Levene variance homogeneity test in this study is 0, less than 0.05, that is, not all dependent variables in any classification of independent variables have equal variance.

When the sample size of each group was close to the same or the same, the homogeneity of variance analysis showed some robustness. That is, only when the sample size of each group is the same, the influence of variance homogeneity on the result of the difference analysis is greatly reduced. At this time, with the increase of sample size, the influence will be further reduced.

In this study, the sample size is the same and conform to the normality distribution. Then we believe that even if the variance is not homogeneous, we can also try to use the three-factor ANOVA model.

Analysis of interaction effects

In this part, first step is to determine whether there is a three-factor interaction. The main purpose of three-factor ANOVA is to determine whether there is three-factor interaction. Three-factor interaction refers to that the interaction of two factors has different effects on dependent variables at different levels of another factor. In this study, SPSS output results are as follows:

Tests of Between-Subjects Effects					
Dependent Variable: Germination rate					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	71009.838 ^a	31	2290.640	7.604	.000
Intercept	169329.668	1	169329.668	562.095	.000
Species	40533.372	1	40533.372	134.552	.000
Depth	5805.363	3	1935.121	6.424	.001
Light	9956.597	3	3318.866	11.017	.000
Species * Depth	3259.066	3	1086.355	3.606	.016
Species * Light	1793.017	3	597.672	1.984	.122
Depth * Light	3102.816	9	344.757	1.144	.340
Species * Depth * Light	6559.606	9	728.845	2.419	.016
Error	28919.753	96	301.247		
Total	269259.259	128			
Corrected Total	99929.591	127			

a. R Squared = .711 (Adjusted R Squared = .617)

Figure 9: result of test of three-factor interaction

In the Tests of Between-subjects Effects table, if $P > 0.05$, the interaction term is not statistically significant. As highlighted in the table (figure 9), Species*Depth*Light shows that the interaction of the three factors is not statistically significant. That's mean, there is no three-factor interaction.

When there is no three-factor interaction, it is necessary to judge whether there is a two-factor interaction.

The table of Test of between-subjects Effects shows the Effects of three interaction items of two factors on dependent variables. In the table of Test of between-subjects Effects, when $P < 0.05$, the influence of interaction term on dependent variable has statistical significance. When $P > 0.05$, the influence of the interaction term on the dependent variable was not statistically significant.

As highlighted in the figure below (figure 10), the Species*Depth has no statistical significance for the dependent variable, the Species*Light has no statistical significance for the dependent variable, and the Depth*Light has no statistical significance for the dependent variable. There is no two-factor interaction. The analysis of variance is end. And the next thing needs to consider is the effects of each sample factor.

Tests of Between-Subjects Effects					
Dependent Variable: Germination rate					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	71009.838 ^a	31	2290.640	7.604	.000
Intercept	169329.668	1	169329.668	562.095	.000
Species	40533.372	1	40533.372	134.552	.000
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Depth * Light	3102.816	9	344.757	1.144	.340
Species * Depth * Light	6559.606	9	728.845	2.419	.016
Error	28919.753	96	301.247		
Total	269259.259	128			
Corrected Total	99929.591	127			

a. R Squared = .711 (Adjusted R Squared = .617)

Figure 10: result of test of two-factor interaction.

The effect of light and depth on germination

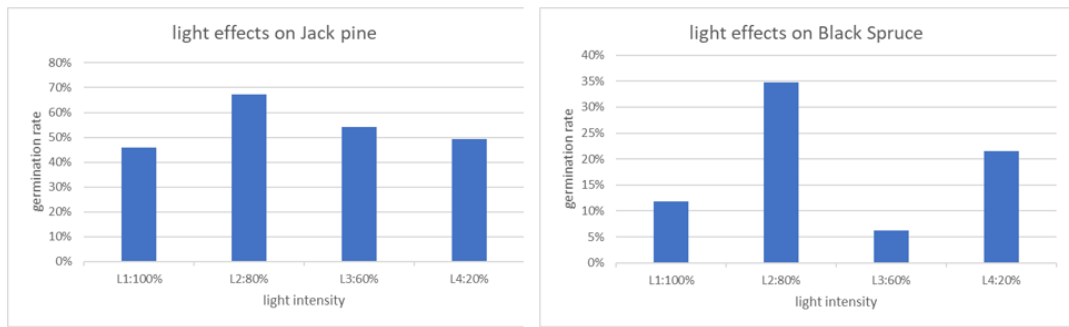


Figure 11: germination rate under different light intensity

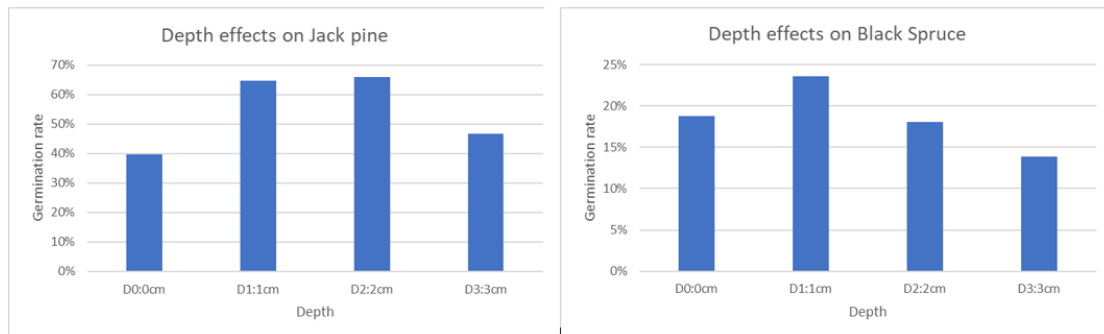


Figure 12: germination rate under different depth

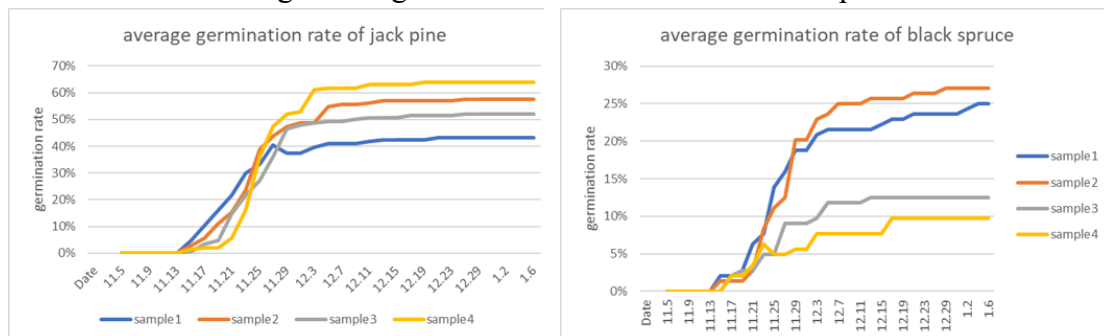


Figure 13: Average germination rate

The results of germination tests showed that light had a significant effect on germination of both Jack Pine and Black Spruce. For Jack pine, in general, the germination rate increased with the increase of light intensity and reached the maximum at about 80% of light intensity. More than 80% of the light intensity, will cause a negative impact on the germination of seeds, reduce the germination rate of seeds. This is in line with Jack pine's need for full light growth. And too much light affects soil moisture, which affects photosynthesis, respiration and transpiration of seeds. For Black

Spruce, in general, it also reached the maximum germination rate at about 80% light intensity. And 20% light intensity followed by 100% light intensity followed by 60% light intensity. Under the same light conditions, the average germination rate of Jack pine is higher than Black Spruce. The average germination rate of Jack pine is about 50~70%; the average germination rate of Black Spruce is about 5~35%. For light, after comparing between groups, it was found that the germination rate was higher under 80% light intensity.

Depth also has a significant effect on germination both Jack pine and Black Spruce. For Jack pine, the germination rate increased with depth increase of depth, and reached the maximum at about 2cm. After 2cm, the germination rate decreased with the increase of depth. For Black Spruce, the germination increased with decrease of depth and reached the maximum at 1cm. After 1cm, the germination rate decreased with the decrease of depth. Also, under the same depth, the average germination rate of Jack pine is higher than Black Spruce. The average germination rate of Jack pine is about 40~66%; the average germination rate of Black Spruce is about 14~24%. Under the same planting depth conditions, for each species, the impact is different. And after comparing between groups, the optimum planting depth is different for each species

In general, in the same conditions, Jack pine germinated after 8 days later and Black Spruce germinated after 17 days later. However, the germination period of Jack pine was concentrated in 8 to 30 days after planting; The germination period for Black Spruce is shorter, concentrating between 17 and 26 days after planting. By contrast, the germination of Black Spruce was later and shorter than that of Jack pine. Compare light with depth, light have more effects on seed germination. Compare with species, Jack pine germinate easier.

Acknowledgements

The author gratefully acknowledges Dr. Wang and Miss Keri Pidgen for their advice, and Dr. Wang for his help with statistical analyses. I also thank Miss Keri Pidgen for her help in green house.

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What I need in this paper is the discussion part, it gives me some example and method.

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Appendices

Table1: Designed table for data

L	D	Black Spruce				Total	D	L	Jack Pine				Total	D	L
		1	2	3	4				1	2	3	4			
1	0	1	2	2		5	27	17	3	2	1	4	10	57	66
2	0	4	6	4	6	20			3	4	3	6	16		
3	0	1				1			3	6	3	3	15		
4	0	1				1			3	3	4	6	16		
1	1	2	1			3	34	50	4	3	5	6	18	93	97
2	1	1	3	3	2	9			6	9	6	9	30		
3	1	3	2			5			2	9	5	9	25		
4	1	6	5	3	3	17			3	9	4	4	20		
1	2	3	4	1		8	26	9	5	7	4	7	23	95	78
2	2	3	4			7			5	9	6	9	29		
3	2	2	1			3			4	4	6	7	21		
4	2	3	3	2		8			6	5	7	4	22		
1	3	1				1	20	31	4	2	4	5	15	67	71
2	3	3	6	2	3	14			4	3	7	8	22		
3	3					0			4	6	4	3	17		
4	3	2	2	1		5			3	2	6	2	13		
Total		36	39	18	14	107			62	83	75	92	312		

Table 2: Table of germination rate calculation

L	D	Black Spruce				Average	D	L	Jack Pine				Average	D	L
		1	2	3	4				1	2	3	4			
1	0	11%	22%	22%	0%	14%	19%	12%	33%	22%	11%	44%	28%	40%	46%
2	0	44%	67%	44%	67%	56%			33%	44%	33%	67%	44%		
3	0	11%	0%	0%	0%	3%			33%	67%	33%	33%	42%		
4	0	11%	0%	0%	0%	3%			33%	33%	44%	67%	44%		
1	1	22%	11%	0%	0%	8%	24%	35%	44%	33%	56%	67%	50%	65%	67%
2	1	11%	33%	33%	22%	25%			67%	100%	67%	100%	83%		
3	1	33%	22%	0%	0%	14%			22%	100%	56%	100%	69%		
4	1	67%	56%	33%	33%	47%			33%	100%	44%	44%	56%		
1	2	33%	44%	11%	0%	22%	18%	6%	56%	78%	44%	78%	64%	66%	54%
2	2	33%	44%	0%	0%	19%			56%	100%	67%	100%	81%		
3	2	22%	11%	0%	0%	8%			44%	44%	67%	78%	58%		
4	2	33%	33%	22%	0%	22%			67%	56%	78%	44%	61%		
1	3	11%	0%	0%	0%	3%	14%	22%	44%	22%	44%	56%	42%	47%	49%
2	3	33%	67%	22%	33%	39%			44%	33%	78%	89%	61%		
3	3	0%	0%	0%	0%	0%			44%	67%	44%	33%	47%		
4	3	22%	22%	11%	0%	14%			33%	22%	67%	22%	36%		
		25%	27%	13%	10%	19%			43%	58%	52%	64%	54%		