EFFECTS OF LALRISE VITA. BACILLLUS VELEZENSIS ON PINUS BANKSIANA AND PICEA MARIANA

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Major Advisor Second Reader

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ABSTRACT

Lachine, A. 2022, Effects of Lalrise Vita. *Bacillus velezensis* on *Pinus banksiana* and *Picea mariana*. 39 pp.

Key Words: *Bacillus velezensis*, Black spruce (*Picea mariana*), forest regeneration, growth potential, Jack pine (*Pinus banksiana*)

Jack pine, *Pinus banksisa*na, and black spruce, *Picea mariana*, are the top two species harvested and planted in the boreal forest of Canada. Sustainable forest management incorporates greenhouses and artificial fertilizers that are used to produce high-quality seedlings to regenerate the forest. The purpose of this thesis was to determine if the growth-promoting rhizobacteria, *Bacillus velezensis,* would increase the height, root collar, shoot, and root mass of these seedlings, and optimize artificial fertilizers uses in greenhouse production. Both tree species had three treatments that were compared to each other to determine the growth differences. Treatment one was comprised of the controls, treatment two had three applications of the microbe, and treatment three had only two applications of the microbe. Each treatment had four replicate trays randomly placed on the greenhouse bench. A one-way ANOVA was run for each variable being measured with a statistical significance P-value <0.05 to determine if the addition of *Bacillus velezensis* had significant differences from the control. The results showed that there were no significant differences in the height, root collar, and mass of jack pine or black spruce seedlings inoculated with the seedlings, and therefore, would not be a good alternative to artificial fertilizers in greenhouse production. The Lakehead greenhouse, where this trial took place, had other trials with a variety of plants along both sides of the bench. These plants were greater in height and some carried diseases which could have affected the results. However, jack pine and black spruce are tree species that are adapted to succeed well in nutrient-poor and harsher conditions. They also evolved to be slow-growing species in the forest succession process. These two mechanisms may contribute to why the Lalrise Vita did not significantly increase the growth of the jack pine and black spruce over a five-month period.

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AWKNOWLEDGEMENTS

I want to thank Rhoda DeJonge, from Lallemand Inc, for being the second reader and for her guidance throughout the project. I want to thank Keri Pidgen-Welyki and Dr. Jian Wang for their support. I want to also thank Lakehead University and Lallemand Inc. for the equipment required to complete this thesis. I'd like to thank Braden Webb, former Lakehead Graduate, for his help in the data collection. Lastly, I want to thank Brian and Cathy Lachine on their endless support.

INTRODUCTION

Canada is covered by a vast amount of forested land covering about 500 million hectares (Madore and Bourdages 1992; Canada Action 2021). Of that, 244 million ha are managed and are an immense contributor to Canada's economy, especially for rural communities, with approximately $1.9 billion in revenue for the provincial and federal government and $33 billion in exports in 2019 (NRC 2020a; Forest Products Association of Canada 2021; EDC 2017; Canada Action 2021). An obligation in forest operations is to regenerate harvested blocks (MacKinnon 1967). Nurseries are used in forestry to produce a large amount of good quality native stock annually to reach this reforestation objective (Agarwal n.d).

Artificial fertilizers are popular in agriculture and forestry to promote growth in their stocks. However, artificial fertilizers can have negative effects on an ecosystem as well as contribute to the climate crisis through the use of fossil fuels (Muscanescu 2013). Naturally occurring alternatives to artificial fertilizers are increasing in interest within research and literature.

Lalrise Vita *Bacillus velezensis* is a rhizobacterial microbe that colonizes the roots of plants within the soil (Lallimand n.d). A symbiotic relationship is formed between the plant and the microbe. The microbe uses the plant to increase the size of its population while the plant benefits because it receives an increase in accessible nutrients for uptake from the increased surface area of *Bacillus velezensis* (Lallemand n.d). *Bacillus velezensis* makes phosphorus in an available form for the plant, which in return promotes growth and tree vigor (Lallemand n.d).

If the microbe can improve the growth of seedlings, then it could be a natural and beneficial alternative to plant growth and the soils ecosystem long term (Muscanescu 2013).

This thesis sets out to explore how the microbe *Bacillus velezensisi* being introduced into the soil of *Pinus banksiana* (Lamb.) and *Picea mariana* (Mill.) seedlings affects their growth. Growth will be measured through the height, root collar, and shoot and root mass weight.

OBJECTIVE

The experiment took place in a controlled greenhouse with three different treatment levels for each species. All seedlings received fertilizer, but two of the treatments also received *Bacillus velezensis*. Treatment one consisted of the control, treatment two had three applications of the Lalrise Vita. *Bacillus velezensis* and treatment three had two applications. By comparing the differences in height growth, root collar growth, and dried mass between the two treatments and the control, the anticipation is to observe increases in plant growth and mass.

HYPOTHESIS

(H_0) There is no difference between the height, root collar, and root mass growth of *Pinus banksiana* and *Picea mariana* inoculated with *Bacillus velezensis.*

(H_1) There is increased height, root collar, and root mass growth of *Pinus banksiana* and *Picea mariana* inoculated with *Bacillus velezensis.*

(H_2) There is decreased height, root collar, and root mass growth of *Pinus banksiana* and *Picea mariana* inoculated with *Bacillus velezensis.*

Will Lalrise Vita *Bacillus velezensis* increase the growth of *Pinus banksiana* and *Picea mariana* and be a good addition to artificial fertilizers use and help mitigate the effects of climate change on regenerating forests.

LITERATURE REVIEW

FORESTRY IN CANADA

The forestry industry within Canada has been acknowledged as "a global leader" for its management practices in the renewable resource of forestry (FPAC 2021; EDC 2017; Canada Action 2021). Forest management that sustains forests long-term has become the main concern within the forestry sector (Madore and Bourdages 1992; MNDMNRF 2021). Within sustainable forest management, there are many requirements that need to be met which include the obligation of reforestation (MNDMNRF 2021). Site preparation mechanisms and replanting efforts are major costs to the forestry companies that hold tenure agreements with the Crown forest (MacKinnon 1967).

The Government of British Columbia (2017) and Ontario stated that all harvested blocks are reforested via natural regeneration, seed, or plant stock (GBC 2017; MNDMNRF 2015b). In British Columbia alone 218 million trees are planted on average a year with 80% of those regeneration efforts being planted stock (GBC 2017).

Grossnickle (2012), Sutherland and Day (1998), and Mohammed et al. (2001) state that having the most successful reforestation program comes down to the quality of the trees planted. Higher quality and larger trees have a better chance of survival in the early stages of recruitment (Grossnickle 2012; Moustakas and Evans 2015; Colomo et al. 2005; Nicholson et al. n.d). Therefore, the goal of reforesting in sustainable management is to find ways to produce the most vigorous trees with higher chances of survival. Often in greenhouse seedling production, bigger and more vigorous seedlings are produced in shorter amounts of time as a result of the application of artificial fertilizers.

BOREAL TREE SPECIES

The boreal forest is made up of trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera*), jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) B.S.P*),* white spruce (*Picea glauca (*Moench), balsam fir (Abies balsamea (L.) Mill), and tamarack (*Larix laricina* (Du Roi, K. Koch) (NRC 2020b).

Black Spruce and Jack Pine

Within the Ontario boreal forest, black spruce species make up the largest part of the land base (MNDMNRF 2021b). Jack pine and black spruce together make up 89.72% of the total 36,600,871.8 ha of forested area in 2021 (MNDMNRF 2021b).

They are also the top two softwoods with the highest net merchantable volume in Ontario (MNDMNRF 2016). In 2016 and 2017, they were also the top two species harvested as shown in Figure 1 (MNDMNRF 2021a).

Figure 1. Volume (m^3) harvested by tree species in Ontario. Source: MNDMNRF 2021a.

Tree species	2016	2017
White pine	374,609	396,285
Red pine	188,188	209,182
Jack pine	3,865,697	3,969,955
Spruce	6,109,522	6,147,912
Balsam fir	510,891	499,720
Other softwood	66,675	42,788
Poplar	2,165,839	2,340,008
White birch	272,473	281,801
Maple	465,533	394,249
Oak	34,660	41,525
Yellow birch	30,249	35,818
Other hardwood	46,102	36,447
Mixedwood	524,048	291,088

The main use of black spruces is pulp and paper but can also be used for dimensional lumber (MNDMNRF 2021b). Black spruce is associated with a range of sites. It is found in wet and organic peat sites, deep sandy soils, shallow tills, and loams and clays (Fryer 2014). Black spruce can maintain on nutrient-poor and dry soils, however, prefer fresh to moist soils with medium nutrients (Fryer 2014).

Jack pine is mainly harvested for the use of dimensional lumber but can also be used for pulp and paper (MNDMNRF 2021b). Jack pine is most commonly associated with sandy soils and rocky outcrops which are both nutrient-poor acidic environments (Carey 1993). They can but rarely persist in thin loamy soil and peatlands (Carey 1993).

As mentioned above, all harvested blocks require to be reforested via natural regeneration, seeding, or plant stock. Black spruce and jack pine are both not recommended to reforest through natural vegetative growth, and therefore nursery stock is often used (MNDMNRF 2015a). Being the top two tree species to be harvested, bigger and more vigorous seedlings for these species produced in shorter amounts of time is ideal.

NURSERIES

Across Canada, there is a large number of nurseries that grow stock to replant a harvested block (CFW 2022). The Forest Products Association of Canada (2021) approximated that 440 million trees are planted every year. Artificial fertilizers are a common application used in forest nurseries to promote growth in their stocks as well as on-site with the stock.

ARTIFICIAL FERTILIZERS

The goal in silviculture for fertilizers is to increase the productivity of trees by increasing the micro-and macronutrients in the soil (NRC 1995). Artificial fertilizers application is the most common form of nitrogen, phosphorus, and potassium for container stocks in forest reforestation (McTavish Resource and Management Consultant LTD 2003; Stoeckeler and Arneman 1960; Zheng et al. 2018). Zheng et al (2018) found that slow-release artificial fertilizers lower the rotation time of these seedlings in the nursery.

Artificial fertilizers are temporary and often require multiple applications, which is costly in both product and labor (Zheng et al. 2018). Fertilizer Canada (2021) also put out a statement about the Federal Government creating a target of reducing emissions from fertilizer by 30%.

Rabbee et al. (2019) wrote "-increased use of chemical fertilizers and pesticides has resulted in the accumulation of residual chemical compounds in the environment, and pathogenic microorganisms are starting to develop resistance." This was outlined because the microbe *Bacillus velezensis* has been increasingly being recognized as a natural growth promoter (Rabbee et al. 2019). With the reduction of fertilizers and the increasing evidence of the negative impacts on the environment, it is good to explore the use of microbes in nursery productions for growth promotion.

RHIZOSHERE

The rhizosphere is the part of the soil where the tree roots interact with the soil through the release of certain acids to obtain nutrients and water to grow (Marks 1973; Barea et al. 2005; Kennedy and Luna 2005). The rhizosphere is also home to a diverse population of natural microbes and fungi (Marks 1973; Barea et al. 2005).

Microbes within the soil can create a mutualistic relationship with the roots rhizosphere (Pandey and Palni 2007; Barea et al. 2005). They have evolved with the ability to aid each other, the rhizosphere being an ideal environment for the microbe to grow and the microbe being an extension for nutrient uptake in the roots present in the rhizosphere (Martin et al. 2017; Pandey and Palni 2007; Bhattacharyya and Jha 201; Backer et al. 2018).

More specifically, plant growth-promoting rhizobacteria's (PGPR) main mechanism is making unavailable nutrients accessible and protecting the plant from other harmful microbes and fungi (Bhattacharyya and Jha 2012; Prasad et al. 2019; Backer et al. 2018; Barea et al. 2005; Rabbee et al. 2019). Indirectly these affect the trees above and below the ground in their growth abilities due to the increased availability of nutrients (Bhattacharyya and Jha 2012; Prasad et al. 2019; Backer et al. 2018).

<u>Lalrise Vita *Bacillus velezensis*</u>

There are many different Genera of Plant Growth-Promoting Rhizobacteria (PGPR). The PGPR that this thesis incorporates is called *Bacillus velezensis.* The family *Bacillus* is a PGPR that specializes in the promotion of plant growth but also can aid in the fighting of negative pathogens (Pindi et al. 2013; Rabbee et al. 2019). It was formally known as being a part of the *Bacillus amyloliquefaciens* family but has since been changed to the above (Meng et al. 2016). This microbe's main characteristic is its ability to produce bioactive secondary metabolites (Rabbee et al. 2019; Pindi et al. 2013). Reva et al. (2004) and Rabbee et al. (2019) state that they can promote growth and suppress fungal invasion through the release of secondary metabolite and the formation of biofilm. It is also being heavily researched as a biological control method for various fungal diseases on a variety of plant species (Meng et al. 2016).

Bacillus velezensis is a gram-positive microbe that can be inoculated into the soil of seedlings (Rabbee et al. 2019; Fan et al. 2018; Lallemand n.d). Once the microbe colonizes the roots, it can start producing the required nutrients for the plant's roots to access and maintains a relationship through the roots life (Rabbee et al. 2019; Xu et al. 2018; Lallemand n.d). *Bacillus velezensis* has come under a spotlight recently in research for it has recently been proven to be advantageous in some hardwood species, crop species, and softwood species.

Torres et al. (2020), conducted a study using the crop species tomato, pepper, pumpkin, and cucumber plants. *Bacillus velezensis,* when inoculated into the soil of these crop species, proved to increase the fresh weights of the species compared to the controls, respectively.

A study on *Bacillus velezensis* and its effects on Sawtooth oak (*Quercus acutissima)* was recently done by Won et al. (2021). They found that *Bacillus* increased the plant's nutrient uptakes and seedling biomass in comparison to the control and chemical fertilizer, respectively.

Cypress (*Chamaecyparis obtusa*) is a softwood species like jack pine and black spruce. Moon et al. (2021) conducted a study on how *Bacillus velezensis* affects the growth of Japanese Cypress. In their study, the seedling that received the treatment of *Bacillus velezensi* resulted in a "-significant increase in the growth of *C. obtusa* seedlings compared to control, or chemical fertilizer treatment, respectively" (Moon et al. 2021).

Lallemand has conducted studies on black cherry (*Prunus serotina*), eastern larch (*Larix laricina*), silver maple (*Acer saccharinum*), red oak (*Quercus rubra)*, white oak (*Quercus alba)*, ursuian pear (*P. pyrifolia)*, hemlock (*Tsuga canadensis)*, chestnut (*Aesculus hippocastanum)*, almond (*Prunus glandulosa)*, apple trees (*Malus domestica*), apricots (*Prunus armeniaca*), and cypress (*Cupressus nootkatensis*) as well. These trials all showed significant differences between the control and the seedlings inoculated with the microbe when it came to height, root collar, and dried mass weight (Lalleman n.d).

This thesis sets out to explore how this species of *Bacillus* affects the growth of jack pine and black spruce.

MATERIALS AND METHODS

MATERIALS

Rhizobacteria

The Growth Promoting Rhizobacteria *Bacillus velezensisi* was used in this study. A company named Lallemand Plant Care produced a wettable powder version called LALRISE VITA, which was applied to the soil. Two of the treatments had it added to the soil. Treatment two had three applications of 0.1g per seedling. Treatment three had two applications of 0.1g per seeding.

Species Used

The two tree species used in this study were jack pine (*Pinus banksiana*) and black spruce (*Picea mariana*). The seeds were sown in May 2021 and once germination took place were transported to their respective trays. There was a total of 540 jack pine and 540 black spruce to start.

Growing Instruments

Fertilizer and Soil

For nutrients, the slow-release fertilizer Osmocote 18-6-12 was used and added to the soil for the seedlings before seeds were placed.

Trays

The jack pine and black spruce both had three treatments. Each treatment had four replicate trays that held 45 seedlings each. There was a total of nine trays used.

Measuring Instruments

The measuring instruments used were: a ruler, root collar caliper, paper bags, scissors, a marker, a laboratory drying oven, and a scale. The ruler was used for height measurements (cm). The root collar caliper was used to measure the root collar (mm). The scissors were used to separate the roots from the stem. The bags were labeled with a marker to identify the roots and shoots of the trees. Those bags and biomass were dried in the oven at 360ºC. The scale was then used to measure the mass (g) of each bag and biomass.

METHODS

Location

The trial took place in the Lakehead University Greenhouse as shown in figure 1. The study ran between May 2021 when the seeds were first sewn until November 2021when they were measured.

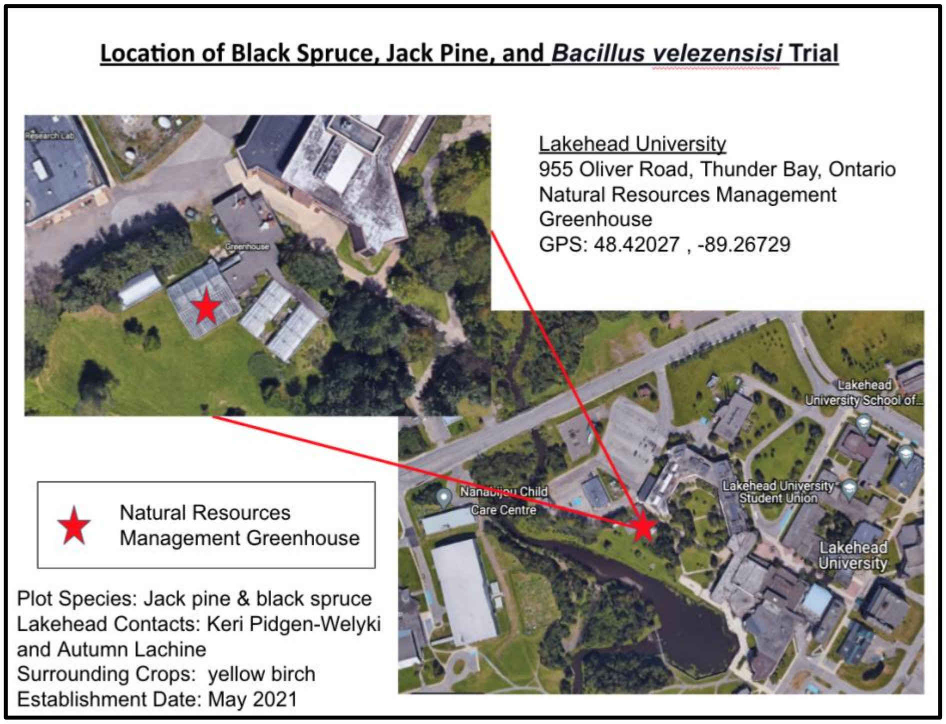


Figure 1. Location of black spruce, jack pine, and *Bacillus velezensisi*

Figure 2. Seedlings before final measurements in their trays randomly placed

Study design and Setup

Seedlings and Placement into Trays

The seedlings used in this trial were sewn in May of 2021. When the seeds germinated they were then randomly placed into trays with moist soil and the slow-release fertilizer.

Each tray was randomly assigned to a treatment and replicate number and was placed at their greenhouse location at random, ensuring each treatment was not grouped together and evenly spaced throughout. Each tray and tree were labeled based on their treatment and replicate, and each tree was numbered individually. Respective trays and their treatment are shown in table 1. The difference in the number of trees before and after for the height and root collar was due to mortality in some of the seedlings.

Table 1. Study design with species, treatments, and their respective information regarding number of trees and trays

Species	Treatments	Replicates	Tray #'s	# of trees before and after for height/ root collar	# of trees for weighed oven-dry biomass
Jack pine	T1: only water	1-4	1-4	180 ->169	40
	T2: 0.1g in water (54g total of LALRISE VITA)	1-4	5-8	180 -> 175	40
	T3: 0.1g in water (36g total of LALRISE VITA)	1-4	9-12	180 -> 177	40
Black spruce	T1: only water	1-4	13-16	180 -> 112	40
	T2: 0.1g in water (54g total of LALRISE VITA)	1-4	17-20	180 ->135	40
	T3: 0.1g in water (36g total of LALRISE VITA)	1-4	21-24	180 -> 158	40

Application of LALRISE VITA

Each tray was watered daily evenly across the trays to moisten the soil. After applications, all materials used were disinfected using bleach. The LALRISE VITA was closed and stored properly between each application.

Treatment One Application.

In Treatment 1, water was the only applicate used. The first step in setting up treatment was filling an 8L clean bucket with water. Each seedling (180 spruce and 180 jack pine) in treatment one was saturated with 40mL of water. This application was repeated once four weeks later, and again after eight weeks. The date of application was recorded and pictures were taken with a ruler for reference at end of the study.

Treatment Two and Three's First and Second Application.

For treatments two and three first application of 0.1g of LALRISE VITA in water was added to each seedling for both jack pine and black spruce. This was done in June 2021 with treatment one.

During the first step, a clean 8L pale was filled with 7.2L of water. 32g of LALRISE VITA was measured on the scale. The water was stirred to create a vortex to which the 32g of LALRISE VITA was then added. 40mL of the solution was measured and added to each treatment with two seedlings (180 jack pine and 180 black spruce). The date of application was recorded and pictures were taken with a ruler for reference at end of the study.

This step was repeated to all treatment three seedlings (180 jack pine and 180 black spruce). All these steps were repeated four weeks after the first June application.

Treatment Two's Third Application.

Four weeks after treatment two's second application, a final one was added. In the first step, a clean 8L pale was filled with 3.6L of water. 18g of LALRISE VITA was weighed out using the scale. The water was stirred to create a vortex to which the 18g of LALRISE VITA was then added 40mL of the solution was measured and added to each treatment with two seedlings (180 jack pine and 180 black spruce) for the final time. The date of application was recorded.

Data Collection and Procedure

For the Data collection portion of this trial, the height (cm) and root collar diameter (mm) were taken for both species across all treatments and replicates. The total number of trees measured for height and root collar was 540 jack pine and 540 black spruce.

The roots and shoots were also measured separately after being oven-dried. Only 10 trees from each tray were selected using a random number generator between 0 and 45. Therefore, 120 black spruce and 120 jack pine were measured. Photos of each replicate from each treatment for both species were taken before any applications and at the time of measurements.

Height.

In November of 2021, the heights were measured and recorded in centimeters. This was done with every seedling for both species from the root collar to the terminal bud. When recording measurements, the species, treatment, its replicate, and tree number were also recorded alongside. Photos were also taken for some seedlings for reference.

Root Collar.

In November of 2021the root collar diameter was measured and recorded millimeters. This was done with every seedling for both species. When recording measurements, the species, treatment, its replicate, and tree number were also recorded alongside.

Weights of Roots and Shoots.

Once both height and root collar was measured for all seedlings destructive sampling and weighing took place. For each tray, 10 trees were taken based on 10 numbers chosen using a random number generator. Therefore, for each treatment, there were 40 samples with a total of 120 jack pine and 120 black spruce.

For each seedling chosen, they were taken out of their tray cell and the soil was washed off the roots. Once the roots were carefully washed, the roots and shoots were separated at the root collar and put in paper bags. The paper bags were labeled with species, treatment, tray, replicate, and whether it was root or shoot.

After the roots and shoots of the 240 seedlings were bagged they were put in an oven and dried for two days. Each bag was weighed and recorded. When recording the weights of the roots and shoots, the species, treatment, its replicate, and tree number were also recorded. Photos were also taken of the bare-root seedlings for reference.

Statistical Analysis

All results were recorded within allotted tables within excel. To test the significance of each treatment's growth an SPSS One-way ANOVA was run using the significance level of 0.05. This was run for the height, root collar, and roots vs shoot weight. ANOVA's were also done between the same replicates in each treatment. Hochberg's post-hoc test was performed on the variables that showed significant differences between the trays within the same treatment to remove the significantly different ones.

RESULTS

As mentioned above, this thesis sets out to explore how *Bacillus velezensis* being introduced into the soil of jack pine and black spruce seedling*s* affects their growth. The three treatments are the control, three applications of the *Bacillus* product, and two applications of the bacillus product. The differences in height growth, root collar growth, shoots, and root dry weights were determined using SPSS's One-way ANOVA with a significance level of 0.05.

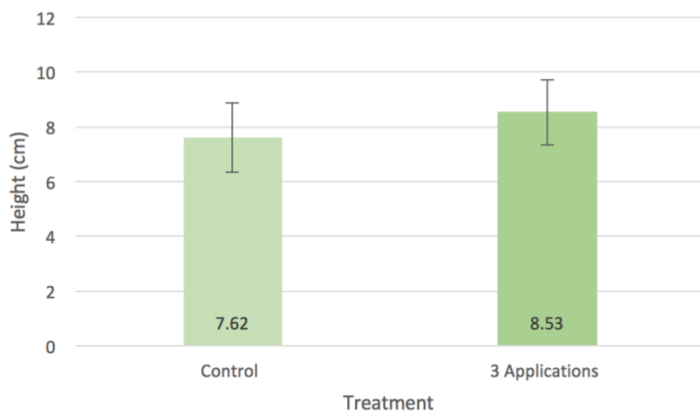
JACK PINE

Each treatment's four trays were individually analyzed to identify if there were significance between the trays within the same treatment. An ANOVA comparing the three treatments displayed that there were significant differences in all three treatments. A post-hoc test determined that a tray in treatment one was significantly different, which was removed. A post-hoc also determined that a tray in both treatments one and two was significantly different from the others, and therefore theses trays were removed from further analysis. The post-hoc for treatment three showed that all trays were significantly different from each other and therefore all of treatment three was removed from the final analysis. Final results are below.

<u>Average Height</u>

The mean height of the control trees was 7.62 cm and the seedlings with three applications were 8.53cm (Figure 4). The treated seedlings had a greater mean height than the untreated seedlings, numerically. The ANOVA test results displayed there was a significant difference with a p-value of <0.05 ($<.001$). However, it was the only significant growth variable.

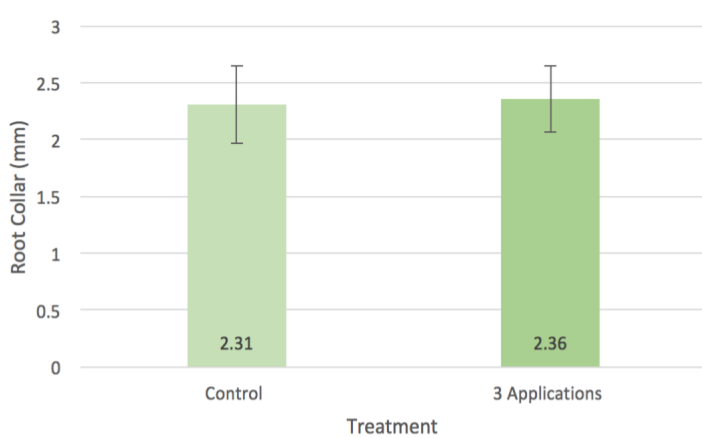
Figure 4. Jack pine height (cm) between



Average Root Collar

The mean root collar for the control trees was 2.31mm and for the seedlings with three applications was 2.36mm (Figure 5). The treated seedlings numerically had a greater mean root collar than numerically than the untreated seedlings. The ANOVA test results indicated there was a not significant difference with a p-value of <0.05 (.238).

Figure 5. Jack pine root collar (mm)



Dry Weight Roots: Shoots

The mean dry-weight for roots of the control trees was 0.61g, and for the seedlings with three applications were 0.58g (Figure 6). The untreated seedlings numerically had the highest mean dried root weight. The ANOVA test results indicated there was a not significant difference with a p-value of <0.05 (.479).

The mean dry-weight for shoots of the control trees was 0.91g, and for the seedlings with three applications were 0.98g (Figure 7). The treated seedlings numerically had the highest mean dried shoot weight. The ANOVA test results showed there was not a significant difference with a p-value of <0.5 (.308).

Figure 6. Jack pine dry weight of roots between

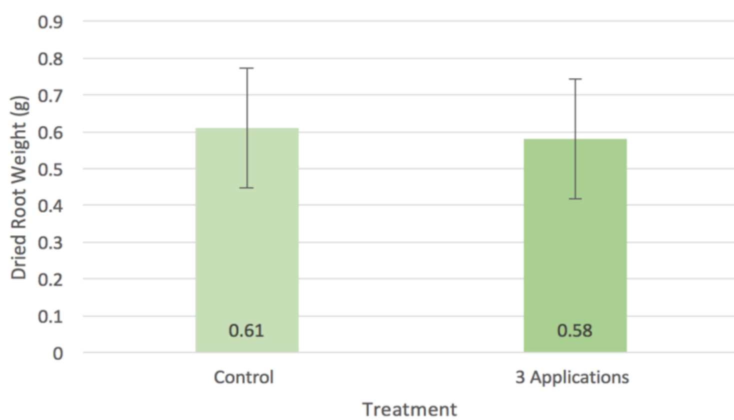
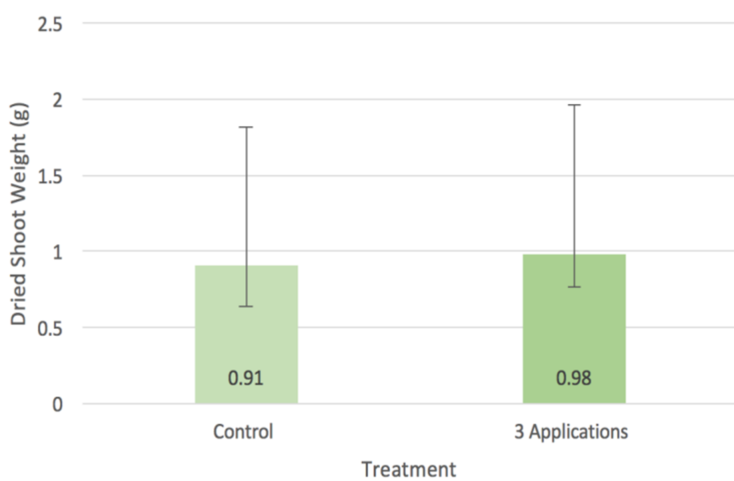


Figure 7. Jack pine dry weight of shoots between



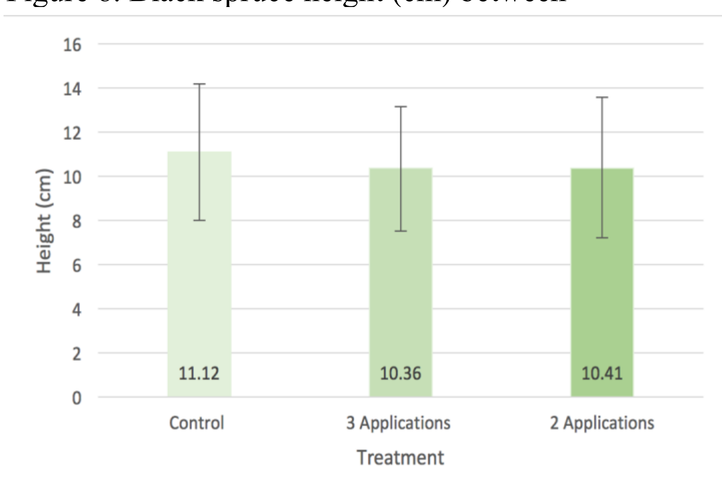
BLACK SPRUCE

Each treatment's four trays were individually analyzed to identify if there were significance between the trays within the same treatment. The ANOVA displayed that there were significant differences in the control and treatment two. A post-hoc test determined that a tray in treatment one was significantly different, which was removed. A post-hoc also determined that a tray in treatment two was significantly different, which was removed. The post-hoc for treatment three showed that all trays were not significantly different from each other and therefore all of treatment three were kept for the final analysis. Final results are below.

Average Height

The mean height for the control trees was 11.12cm, for the seedlings with three applications was 10.36cm, and for the two applications was 10.41cm (Figure 8). The untreated seedling's mean height was numerically higher than the treated seedlings. The ANOVA test results indicated there was a not significant difference with a p-value of <0.05 (.126).

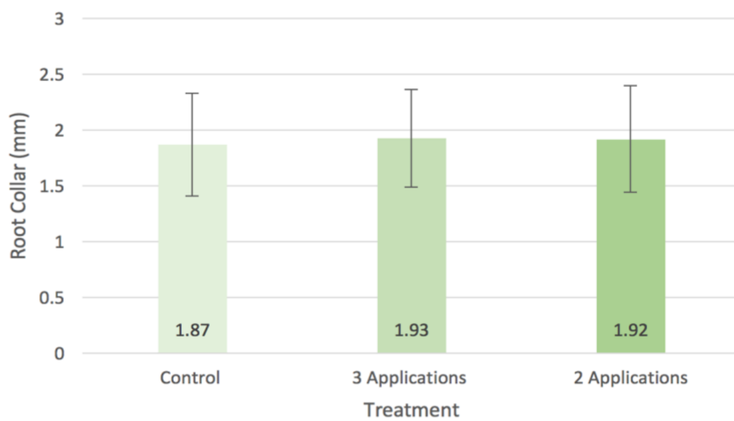
Figure 8. Black spruce height (cm) between



Average Root Collar

The mean root collar for control trees was 1.87mm, the seedlings with three applications were 1.93mm, and the two applications were 1.92mm (Figure 9). The seedlings with three applications had the largest mean root collar (mm) numerically, and the control had the smallest mean root collar (mm). The ANOVA test results displayed there was not a significant difference with a p-value of <0.05 (.483).

Figure 9. Black spruce root collar (mm) between treatments.



Dry Weight Roots: Shoots

The mean dry-weight for roots of the control trees was 0.28g, the seedlings with two applications were 0.25g, and the two applications were 0.26g (Figure 10). The untreated seedlings had a higher mean root dry-weight numerically than the treated ones. The ANOVA test results displayed there was not a significant difference with a p-value of <0.05 (.344).

The mean dry-weight for shoots for the control trees was 0.57g, the seedlings with three applications were 0.62g, and the two applications were 0.56g (Figure 11). The treated seedlings with three applications had a higher mean shoot dry-weight numerically than both the control and other treated seedlings. The three-way ANOVA test results displayed there was not a significant difference with a p-value of <0.05 (.561).

Figure 10. Black spruce dry weight of roots between treatments.

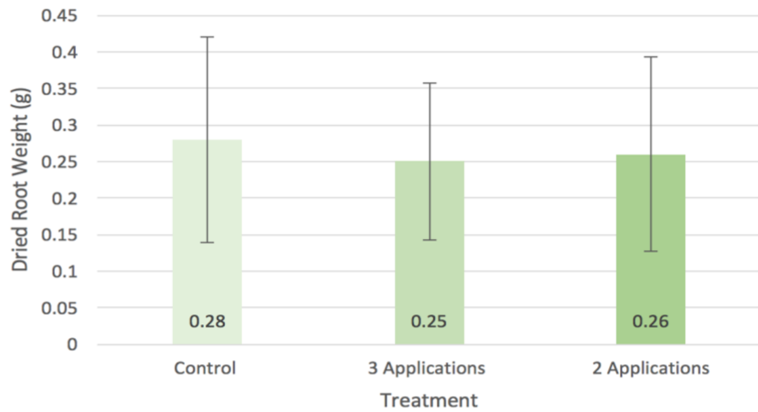
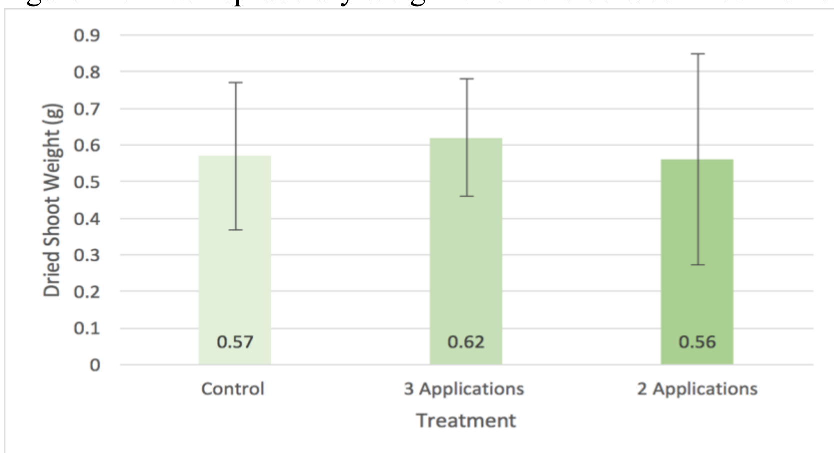


Figure 11. Black spruce dry weight of shoots between treatments.



DISCUSSION

The importance of having increased height, root collar, and mass growth is highly recognized in the replantation part of the forest harvesting process. Having more vigorous seedlings being planted out of the greenhouse prepares the block for higher survivorship and growth targets being met earlier (regeneration/ free-to-grow). The use of naturally occurring growth promotion additives in the greenhouse also leads to fewer artificial fertilizers being used and therefore more cost-effective and less degradation on the environment.

The trial results showed that the jack pine and black spruce did not show significant differences in the growth in their height, root collar, and total dried mass compared to the control in all situations. Resultes were mixed, with the control trees performing better than the treated in some situations, while the treated trees performed better than the control trees in others. Therefore, the inoculation of Lalrise Vita into the soil of jack pine and black spruce did not appear to optimize artificial fertilizers in this greenhouse trial.

For the jack pine's height, the treated seedlings had a greater mean height and a p-value less than <.001. Therefore, the second hypothesis was accepted that there was an increased height growth of the jack pine seedlings inoculated with the microbe. However, this was the only significant result found for jack pine. The jack pine's treated seedlings also had a greater mean root collar and has a p-value of .238. Therefore, the null hypothesis was accepted that there was no difference in root collar growth between the jack pine seedlings inoculated with the microbe and the control.

The jack pine's control had the great mean dried root weight and the p-value was .479. Therefore, the null hypothesis was accepted that there was no difference in root mass growth between the jack pine seedlings inoculated with the microbe and the control. The jack pine's treated seedlings had the greater mean dried shoot weight and the p-value was .479. Therefore, the null hypothesis was accepted that there was no difference in shoot mass growth between the jack pine seedlings inoculated with the microbe and the control.

For the black spruce's height, the control had a greater mean height and have a p-value of .126. Therefore, the null hypothesis was accepted that there was no difference in height growth between the black spruce seedlings inoculated with the microbe and the control. The black spruce's seedlings with three application had a greater mean root collar and has a p-value of .483. Therefore, the null hypothesis was accepted that there was no difference in root growth between the black spruce seedlings inoculated with the microbe and the control.

The black spruce's control had the greater mean dried root weight and the p-value was .344. Therefore, the null hypothesis was accepted that there was no difference in root mass growth between the black spruce seedlings inoculated with the microbe and the control. The black spruce's seedlings with three application had the greatest mean dried root weight and the p-value was .561. Therefore, the null hypothesis was accepted that there was no difference in shoot mass growth between the black spruce seedlings inoculated with the microbe and the control.

The results of this trial for the jack pine and black spruce seedlings do not align with the results shown in other trials done by Lallemand, the Lalrise Vita suppler, and the peer-reviewed findings on crop species. Unlike the trials on silver maple, black cherry, cypress, and eastern larch that show significant differences in the height, root collar, and root mass the jack pine and black spruce don't show the same response.

Jack pine and black spruce, as mentioned in the literature review, are both species that have evolved to compete and excel in nutrient-poor and harsh conditions. The Lalrise Vita, being growth-promoting rhizobacteria, increases the available nutrients within the surrounding soil. Having jack pine and black spruce adapted to nutrient-poor and harsher conditions, the microbe may not be beneficial to them. The trial could have also been too short to be able to see the benefits currently. The microbe may be a good application either way for it is also aids in helping fight off disease, which could be helpful in the field.

There can be a number of reasons why the results turned out as they did. The previous trials were in a more controlled greenhouse without other influences. This trial took place in the Lakehead Greenhouse which had multiple trials occurring at once. On both sides of the bench, which held all the trays of black spruce and jack pine seedlings, were other plants which were taller than the seedlings.

There was a disease in the squash plants that were noted and considered a factor. The seedlings also experienced mortality, which could have been human error or the other outside influences mentioned above. The proximity and shade of the other plants alongside could have played an influence on the seedling's ability to grow on the trays closest. The trays were placed at random and an ANOVA was done to determine and remove certain trays that showed significant differences from the other trays within the same treatment. This removal of certain trays gave more accurate results, however, the trend of increased growth was still not observed.

Therefore, my recommendation going forward, if this trial was to be done again in the future, is to have the trial in a more controlled greenhouse environment. Next, a longer trial that allows more time to grow the seedlings as well as incorporating the growth of the one or two years' post-plant into the field.

The Lalrise Vita. may not be a good alternative to artificial fertilizers, however, could be beneficial in long term alongside fertilizer in the field. This is due to their long-life relationship with the roots throughout the lifespan of the tree.

CONCLUSION

This trial was done to determine if the application of *Bacillus velezensis* into the soil of jack pine and black spruce would result in increased growth in height, root collar, and the root and shoots mass compared to a control. It was also a good start in looking for alternatives to artificial fertilizers. However, the results indicated that the inoculation of the microbe into the soil of both species did not increase the growth significantly and therefore would not be a good alternative to artificial fertilizers. Alone it may not be a good additive, but research into its use of it alongside fertilizers as well as longer-term should be sought after.

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