THE EFFECT OF THE ECTOMYCORRHIZAL FUNGUS *HEBELOMA LONGICAUDUM* ON DROUGHT STRESS OF *PINUS BANKSIANA* SEEDLINGS

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ABSTRACT

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KEYWORDS: Drought, ectomycorrhizal fungi, *Hebeloma longicaudum*, jack pine

This study looks at the influence of the ectomycorrhizal fungus *Hebeloma longicaudum* to form associations and impact growth of jack pine seedlings under simulated drought conditions. Four treatment groups of jack pine seedlings were grown for four months and then data for dry weight, short root count, percentage and colonization of roots was collected for each treatment group. The four treatment groups are as follows; 1. Seedlings inoculated with an ectomycorrhizal fungus and normal watering regime, 2. Seedlings inoculated with an ectomycorrhizal fungus and drought-like watering regime, 3. Seedlings not inoculated with an ectomycorrhizal fungus and normal watering regime, and 4. Seedlings not inoculated with an ectomycorrhizal fungus and drought-like watering regime. Few significant differences were found in the results of this study, however a significant difference was observed in the number of mycorrhizal associations between inoculated and non-inoculated seedlings (>0.01). It is thought that the lack of significant results is primarily due to the mycorrhizal fungus not having enough time to develop associations fully.

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INTRODUCTION

Most woody plant species within the boreal forest and across the globe have symbiotic associations with mycorrhizal fungi (Policelli *et al*. 2020, Averill *et al*. 2019). Mycorrhizal fungi colonize the roots of their host and improve the hosts access to nutrients and water (Stuart and Plett 2020, Policelli *et al*. 2020). In exchange for this, the hosts deliver a portion of their photosynthetic carbon to the fungi (Stuart and Plett 2020). The two main types of mycorrhizal fungi are arbuscular mycorrhizal fungi and ectomycorrhizal fungi. Ectomycorrhizal fungi typically colonize the dominant families in todays forests: Fagaceae, Pinaceae, Betulaceae, and Dipterocarpaceae. Ectomycorrhizal fungi and the associations they form with trees will be the focus of this study.

Ectomycorrhizal fungi present many benefits to trees. They are efficient at acquiring nutrients such as nitrogen (Pena and Polle 2013), phosphorus, magnesium, sulphur, and calcium (Stuart and Plett 2020). Studies have also found that ectomycorrhizal fungi are effective at reducing the effects of heavy metals on adult trees and seedlings (Colpaert *et al*. 2011, Krupa and Kozdroj 2004). Ectomycorrhizal fungi can increase carbon fixation and water uptake while a tree is under drought stress (Park *et al*. 1983). The hyphae of ectomycorrhizal fungi transport water to the tree and act as an extension of the tree's roots (Lehto and Zwiazek 2011). These benefits that the fungi provide to trees will become increasingly important as drought-stress increases in trees globally.

Anthropogenic climate change is causing abnormal fluctuations in the average climate across the world. The average global temperature has risen 1° Celsius since the pre-industrial era (Lindsey and Dahlman 2022). The average temperature of the globe has a

direct impact on the amount of precipitation that occurs as higher air temperatures lead to greater evaporation rates and an increased water holding capacity of the air (about 7% per °1 Celsius of warming) (Trenberth 2011). According to Trenberth (2011) wet areas will receive more precipitation while drier areas will receive less precipitation. The timing of precipitation events will change and there will be significant differences in distribution (Price *et al*. 2013). This will put dry forest areas at increased risk for drought.

Under drought stress, tree growth is altered, photosynthesis rates are reduced, and carbon allocation within trees change (Dobbertin 2005). A run of unusually dry years in a row can lead to widespread tree death (Frelich *et al*. 2021). In response to this, foresters should always be looking for new ways to mitigate the effects of climate change on forests. The western boreal forest in Canada is where most of our lumber exports come from. In 2018 British Columbia produced 45% of Canada's total softwood lumber production (Barnes 2019). British Columbia plants on average 218 million seedlings annually (Government of British Columbia 2017) in order to reforest the harvested timber. Seedlings that have just been transplanted are at an increased susceptibility to drought stress due to roots only being in the upper layers of the soil (Douglas 2023). A solution to increasing seedling survivability is economically and environmentally important.

The goal of this study is to determine the ability of mycorrhizal fungus associations to mitigate the effects of drought on seedlings. This study was started by the germination of jack pine (*Pinus banksiana* Lamb.) seedlings at the Lakehead University greenhouse. Four treatment groups of seedlings were tested; 1. Seedlings inoculated with an

ectomycorrhizal fungus and normal watering regime, 2. Seedlings inoculated with an ectomycorrhizal fungus and drought-like watering regime, 3. Seedlings not inoculated with an ectomycorrhizal fungus and normal watering regime, and 4. Seedlings not inoculated with an ectomycorrhizal fungus and drought-like watering regime. Seedlings were watered following a specified watering regime for approximately 12 weeks and then they were harvested. The number of short roots and mycorrhizal associations were counted on tree roots. The trees were dried in a drying oven and the dry weight was then measured. Comparisons will be made between the four seedling test groups.

This study will provide further information on the effect of ectomycorrhizal fungus associations on drought mitigation among tree seedlings. It will also indicate the impact of an ectomycorrhizal fungi's role in reducing the effects of drought on planted seedlings. This information could be vital in the future as drought and the number of seedlings planted increases.

OBJECTIVES

1. To determine if an ectomycorrhizal fungus will influence the growth and survivability of jack pine seedlings under drought stress

HYPOTHESIS

The jack pine seedlings that have been inoculated with *Hebeloma longicaudum* (Pers.) Kummer. will exhibit higher levels of growth and greater number of colonized short roots than those seedlings not inoculated.

The jack pine seedlings inoculated with *Hebeloma longicaudum* and subjected to a drought-like watering regime will show greater amounts of growth compared with those seedlings that were not inoculated but subjected to a drought-like watering regime.

LITERATURE REVIEW

Mycorrhizal Fungi

Mycorrhizal fungi are symbiotic fungi that form associations with approximately 80% of terrestrial plant species on Earth (Averill *et al*. 2019, Dighton 2009, Dong *et al*. 2018). Mycorrhizal associations are present in almost all biomes on earth excluding the areas where plants do not grow (Van der Heijden *et al*. 2015). Currently, mycorrhizal fungi are being used in forestry and restoration to improve yield and overcome pollutants on disturbed sites (Dighton 2009).

The two types of mycorrhizal fungi that plant species typically form associations with are ectomycorrhizal fungi and arbuscular mycorrhizal fungi. Some plant families such as Fagaceae, Salicaceae, and Myrtaceae can form mycorrhizal associations with both arbuscular mycorrhizal fungi and ectomycorrhizal fungi simultaneously. Arbuscular mycorrhizal fungi (phylum Glomeromycota) associate with plant species with diverse taxa (Toju and Sato 2018) and form associations with approximately 80% of vascular plants (Siddiqui and Pichtel 2008). Ectomycorrhizal fungi (phyla Ascomycota and Basidiomycota) associate with many of the dominant plant families in today's forests such as Fagaceae, Pinaceae, Betulaceae and Dipterocarpaceae (Toju and Sato 2018). These two types of fungi use different nutrient acquisition strategies; arbuscular mycorrhizal fungi acquire nutrients when they are released by saprotrophic microbes while ectomycorrhizal fungi mineralize nutrients from organic matter (Dong *et al*. 2018). Because of their nutrient acquisition strategy, ectomycorrhizal fungi can access some forms of organic nitrogen directly (Dong *et al*. 2018, Van der Heijden *et al*. 2015).

This study is focused on ectomycorrhizal fungi and the associations they form with trees. Ectomycorrhizal fungi are hosted on approximately 6000 or 2% of plant species on Earth. Within this category of mycorrhizal fungi there are around 20 000 different species (Van der Heijden *et al*. 2015). Ectomycorrhizal fungi play an important part in global forest dynamics as they promote the dominance of the species that they form associations with (Toju and Sato 2018). When ectomycorrhizal fungi forms associations with plants they form three distinct pseudo-tissues (Frank and Garcia 2021). The first tissue is extraradical hyphae which searches through the soil for water and acquires nutrients. The next type of tissue forms a mantle which surrounds the roots of the plant species. This mantle surrounds the roots and isolates them from the soil (Frank and Garcia 2021). The final tissue type is a Hartig net which is a network of hyphae where nutrient exchanges take place (Frank and Garcia 2021). The Hartig network is what make ectomycorrhizal fungus an effective symbiotic partner to tree species.

A symbiotic relationship is an association between two species where one is considered a host and there is an exchange of energy or material (Overstreet and Lotz 2016). The type of symbiosis that ectomycorrhizal fungi create with trees is mutualistic which means both organisms benefit (Overstreet and Lotz 2016). Pine species rely on this symbiotic relationship to thrive in natural environments (Aucina *et al*. 2007). Ectomycorrhizal fungi receive photosynthetic carbon from their plant hosts in exchange for higher nutrient and water acquisition (Stuart and Plett 2020). Plants that are hosts for ectomycorrhizal fungi transfer 23% more of their carbon belowground compared to plants that don’t have associations formed (Moore *et al*. 2015).

Benefits to Trees by Ectomycorrhizal Fungi

One benefit provided by the ectomycorrhizal fungi to plant species is the acquisition of nutrients. Plant species in colder regions such as the boreal forest have been found to be mainly dependent on organic nitrogen for nutrients (Kranabetter *et al*. 2009) but nitrogen is a limiting factor in many forested regions (Pena and Polle 2013). Ectomycorrhizal fungi play a key role in nitrogen cycling in boreal regions and in nitrogen acquisition for trees (Pena and Polle 2013). Ectomycorrhizal fungi uptake macronutrients including phosphorus, magnesium, sulphur, and calcium (Stuart and Plett 2020). They also provide micronutrients to trees such as iron, copper, manganese, and zinc (Stuart and Plett 2020). Recent studies have shown that elemental potassium is also transported through the fungi to the host plant. Typically, a lack of potassium in the soil can limit the growth of plant species (Frank and Garcia 2021). Ectomycorrhizal fungi also secrete enzymes that break down organic compounds which can free organic nitrogen and phosphorus, increasing their availability for plant uptake (Qu *et al*. 2010).

Another benefit that ectomycorrhizal fungi provide to their host plants is protection from the accumulation of heavy metals in the soil (Colpaert *et al*. 2011, Tam 1995). Colpaert *et al*. (2011) found that pine seedlings inoculated with metal-tolerant ectomycorrhizal fungi had lower metal concentrations in their needles than seedlings not inoculated. In an area where soils had high heavy metal presence, ectomycorrhizal fungi associated with birch trees were found to fix the heavy metals. They do this by forming a barrier which limited the movement of the heavy metals into the birch tissues (Krupa and Kozdroj 2004). A study done by Tam (1995) found that certain species of

ectomycorrhizal fungi were able to withstand high concentrations of the heavy metals iron, aluminum, copper, and zinc.

Ectomycorrhizal Fungi and Tree Associations and Drought

A major benefit that ectomycorrhizal fungi provide to trees is the uptake of water (Stuart and Plett 2020). There is a lot of research on the subject but limited understanding of their specific role. The external mycelium of ectomycorrhizal fungi can transport water to the tree that it is associated with and essentially act as an extension of the tree's roots (Lehto and Zwiazek 2011). Duddridge *et al*. (1980) found that the minimum rates for translocation of water from hyphae strands to roots was 27 cm h^{-1} which is similar to rates of transport in xylem. Water uptake by hyphae becomes especially important when the soil dries and water remains in smaller pores where only hyphae can reach (Lehto and Zwiazek 2011). A study done by Dixon *et al*. (1980) found that white oak (*Quercus alba* L.) seedlings put under drought stress and inoculated with ectomycorrhizal fungi had larger leaf areas and longer roots than those not inoculated. Another study was done with Douglas-fir seedlings inoculated with ectomycorrhizal fungi, while some were not inoculated and submitted them to drought like conditions. It was found that seedlings under drought stress that had been inoculated with ectomycorrhizal fungi fixed carbon dioxide at a rate ten times more than trees under the same conditions that had not been inoculated (Park *et al*. 1983). Aryal *et al*. (2021) observed that American chestnut (*Castanea dentata* (Marsh.) Borkh.) seedlings inoculated with ectomycorrhizal fungi recovered faster from experimental drought than those not inoculated. Mycorrhizal inoculation was found to increase the growth of radiata pine in drier areas, especially their above ground growth (Ortega *et al*. 2004). A

study by Swaty *et al*. (2004) found that trees on sites with high mortality had 50% less mycorrhizal associations than trees which survived drought on sites with low mortality.

A study done on Virginia pine (*Pinus virginiana* Mill.) seedlings found that mycorrhizal associations formed on the seedlings decreased with the lowering availability of soil water (Worley and Hacskaylo 1959).

Increase of Drought

The increase of average global temperature has become more noticeable over the past decade. The rise is due to the increase in concentrations of greenhouse gasses in our atmosphere (Adams 1989). Since the pre-industrial era, atmospheric carbon dioxide levels have reached 414 ppm (a 48 % increase), atmospheric methane levels have more than doubled, and nitrous oxide levels have increased by 20% since the 1920s (United States Environmental Protection Agency 2022). The increases of greenhouse gasses in the atmosphere lead to the warming of the surface of the earth.

Despite the rise in global temperature only being 1 degree Celsius (Lindsey and Dahlman 2022) the effects are adverse and noticeable. An increase in atmospheric air temperature by 1 degree Celsius causes the water holding capacity of the air to increase by 7% (Trenberth 2011) and there is potential for a further increase of 2 to 4 degrees Celsius even when looking at conservative possibilities (Allen *et al*. 2010). This is expected to push our globe into an overall more arid climate (Seager *et al*. 2007). Warming of the globe in all seasons is expected to amplify evaporation losses as the surface of the Earth increases in temperature (Cook *et al*. 2018). Higher latitudinal areas such as Canada will experience the greatest increase in average temperatures (Brecka *et*

al. 2018). An increase in atmospheric temperature will lead to more precipitation falling in the form of rain rather than snow (Trenberth 2011). This will lead to an increased risk of flooding in the spring and less precipitation during the summer months (Trenberth 2011). Many areas in Canada's west boreal region have experienced strong drying and exceptional droughts in the past couple of decades (Price *et al*. 2013). Numerous models have predicted that there will be an increase in the frequency and intensity of extreme events such as drought (Price *et al*. 2013).

The Effect of Drought on Tree Growth

Drought is increasingly causing tree mortality in forest ecosystems across the globe (Senf *et al*. 2020, Allen *et al*. 2010, Sanchez-Pillinos *et al*. 2021). Drought is one of the most limiting factors to a trees growth and was found to reduce the growth of all dominant tree species (Adams and Kolb 2005). Under drought stress, growth is altered, photosynthesis rates are reduced, and carbon allocation within trees change (Dobbertin 2005). The main physical effect of drought on trees is the damage to the roots, primarily the feeder roots. These roots are typically located within the top 40 cm of soil and shrivel up, becoming non-functional, when drought occurs (Douglas 2023). When long term drought occurs, trees react by closing their stomata to minimize water losses, this leads to a decrease in the photosynthetic uptake of carbon (Sanchez-Pillinos *et al*. 2021). A study done by Sanchez-Pinillos *et al*. (2021) found that frequent low intensity droughts had more adverse effects on boreal forest mortality than one high intensity drought. This was more noticeable in forests dominated by conifers than in mixed wood forests (Sanchez-Pinillos *et al*. 2021). Tree growth appears to be most sensitive to multiannual droughts (Mendivelso *et al*. 2014). Sultana *et al*. (2021) found that three

species of tree seedlings grown under a 25% soil water content showed significant reductions in total dry biomass, survivability, and germination percentage than the tree seedlings grown at both 100% and 50% soil water content.

Drought has the most severe effects on seedlings or trees which have been newly transplanted. This is because the roots of these trees only occupy the upper layers of soil where the soil dries out the quickest (Douglas 2023). Drought causes an overall decline in the health of many woody species and in turn can lead to secondary negative effects. Weakened trees are opened to other issues including winter injury, pathogens, or insect infestation (Douglas 2023).

Water stress not only influences tree health but also the environment that they grow in further increasing negative effects. Lack of water can affect the chemical, physical, and biological activities of soil (Al-Kaisi 2017). Drought leads to changes in both the fungal and bacterial communities of soil. A study done by Chodak *et al*. (2015) found that drought stress altered the soil bacterial community in forest soils. Large changes in diversity and composition of fungal communities are also observed after extensive drought (Buscardo *et al*. 2021).

Importance of Study

As the effect of drought due to anthropogenic climate change continues to plague global forests a solution is needed to counteract this and increase tree survivability. The boreal forest covers 1.9 billion hectares worldwide (Government of Canada 2020) and stores approximately 32% of the worlds forest carbon (Gauthier *et al*. 2015). This forest type is significant in Canada in terms of social, economic and ecological values. The boreal

forests of Canada cover 552 million hectares which is 28% of the global boreal zone (Government of Canada 2020).

The dominant species of trees present in the boreal forest are pine, spruce, larch, and fir (*Pinus*, *Picea*, *Larix*, and *Abies*) (Kayes and Malik 2020). The prevalence of ectomycorrhizal fungi in relation with these tree species means that they may play a critical role of in the restoration of forest ecosystems in the future (Policelli *et al*. 2020). The presence of ectomycorrhizal fungi in the boreal forest allows for plants to have greater growth in the harsh conditions of the boreal forest (Qu *et al*. 2010). Approximately 95% of short roots in the boreal region have associations with ectomycorrhizal fungi (Frank and Garcia 2021) and these fungi play an important role in nutrient cycling and composition (Frank and Garcia 2021). The boreal forest has a low mineralization rate which results in a low availability of nutrients (Qu *et al*. 2010) and the ability of ectomycorrhizal fungi to enhance nutrient uptake for plant species is key to their success. Most importantly ectomycorrhizal fungi can increase their host plants access to water through hyphae that extend into the soil (Lehto and Zwiazek 2010). These fungi can be used to increase the survivability of seedlings planted in a drought. The inoculation of ectomycorrhizal fungi can improve soil quality and assist the establishment of plant species in degraded environments (Policelli *et al*. 2020).

The boreal forest region is crucial to the global timber products market, approximately 25% of paper and 55% lumber global exports are from the boreal forest. Canada has 28% of the worlds boreal forest (Government of Canada 2020) and 94% of that is publicly owned and available for management (Government of Canada 2021). The majority of lumber exported from Canada comes from British Columbia (BC), in 2018

BC produced 45% of Canadas total softwood lumber production (Barnes 2019). Canada relies on British Columbia's forests not only economically but also for mitigation of climate change as forests, especially old growth, can be used as carbon storage (Whitehead 2011, Luyssaert 2008). Unfortunately, the forests on the west side of Canada are at risk of experiencing severe, tree-killing droughts partially due to changes in precipitation patterns from climate change (Price *et al*. 2013). In 2021 excessive foliage damage due to drought occurred across 108,345 hectares of BC forest and drought induced mortality affected 558 hectares of forest. (Westfall and Duthie-Holt 2021). Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and lodgepole pine (*Pinus contorta* Douglas ex Loudon) were among the species most damaged (Westfall and Duthie-Holt 2021) and are two of the species most planted in BC reforestation efforts.

When harvesting occurs in the boreal forest, the companies that are conducting the operations have a responsibility to replant. However, if drought continues to increase, the seedlings that are replanted may struggle to survive and forests will take longer to grow. A solution to this problem may be the inoculation of ectomycorrhizal fungi into seedling plugs before they are planted. These fungi will give the seedlings (which may have had their roots damaged by improper handling or sun exposure) a leg up in the natural environment as they struggle to compete against grasses and weedy plants.

MATERIALS AND METHODS

Research Set-Up

To start the study 100 jack pine seeds were germinated in a flat in the greenhouse on October 5th, 2022, in a covered tray. The seeds had been stored in a freezer prior to germination. The seedlings were allowed to grow in their tray for 44 days (October 13th to November 17th, 2022) and were watered when needed to keep the soil saturated. Dr. Hutchison had grown 5 cultures of the ectomycorrhizal fungus species *Hebeloma longicaudum*. in Petri dishes containing modified Melin-Norkrans agar which were incubated in the dark at 20° C. These cultures were created on October 6th, 2022 and were used to create the mycelial plugs for inoculation of the jack pine seedlings and a photo of them can be seen below in Figure 1a.

Figure 1. (a) The five cultures of *Hebeloma longicaudum* used to create plugs for seedling inoculation; (b) Cork borer used to make plugs for inoculation

On November 17th, 2022, the plugs for the ectomycorrhizal fungus inoculation of seedlings were created. This was completed in the transfer hood in the mycology lab at Lakehead University. The counter surface was sterilized with 70% ethanol before any agar cutting began.. The tool used for this was a 7 millimetre cork borer and can be seen above in Figure 1b. The cork borer was sterilized with 70% ethanol and the alcohol was burned off with a flame from a gas burner before the plugs were cut. Approximately 30 plugs were made from each Petri dish by pushing the cork borer straight into the colonies on the agar. The Petri dishes were then resealed using parafilm.

After the ectomycorrhizal fungal plugs were made the seedlings were transferred from one tray into forty individual pots on the same day. The forty best seedlings from the hundred germinated were used excluding the ones with only a tap root and the ones with dead or dying needles. The pots were then labeled with masking tape to separate the forty seedlings into four treatments with ten seedlings each. The treatments are as seen below in Table 1.

Table 1. Description of four treatment groups

Treatment Group Number	Description
1	Watered twice a week and inoculated with ectomycorrhizal fungus
2	Watered once a week and inoculated with ectomycorrhizal fungus
3	Watered twice a week and no inoculation of ectomycorrhizal fungus
4	Watered once a week and no inoculation of ectomycorrhizal fungus

The four treatments are as follows: Treatment-1: Inoculated with ectomycorrhizal fungus and watered twice a week; Treatment-2: Inoculated with ectomycorrhizal fungus and watered once a week; Treatment-3: No inoculation of ectomycorrhizal fungus and watered twice a week; Treatment-4: No inoculation with ectomycorrhizal fungus and watered once a week. Once the seedlings were separated into treatments, each seedling in treatments 1 and 2 were inoculated with seven plugs of the ectomycorrhizal fungus.

These plugs were taken from the Petri dishes using a spatula tool which can be seen below in Figure 2.

Figure 2. Spatula tool used to transfer ectomycorrhizal fungus plugs into soil for inoculation

The seven mycelial plugs were placed around the roots of the seedling in a circular pattern. Once treatments 1 and 2 were inoculated, all forty seedlings (4 treatments) received 100 mL of water so all soil started at the same moisture level. The set up of the 40 pots of seedlings can be seen below in Figure 3.

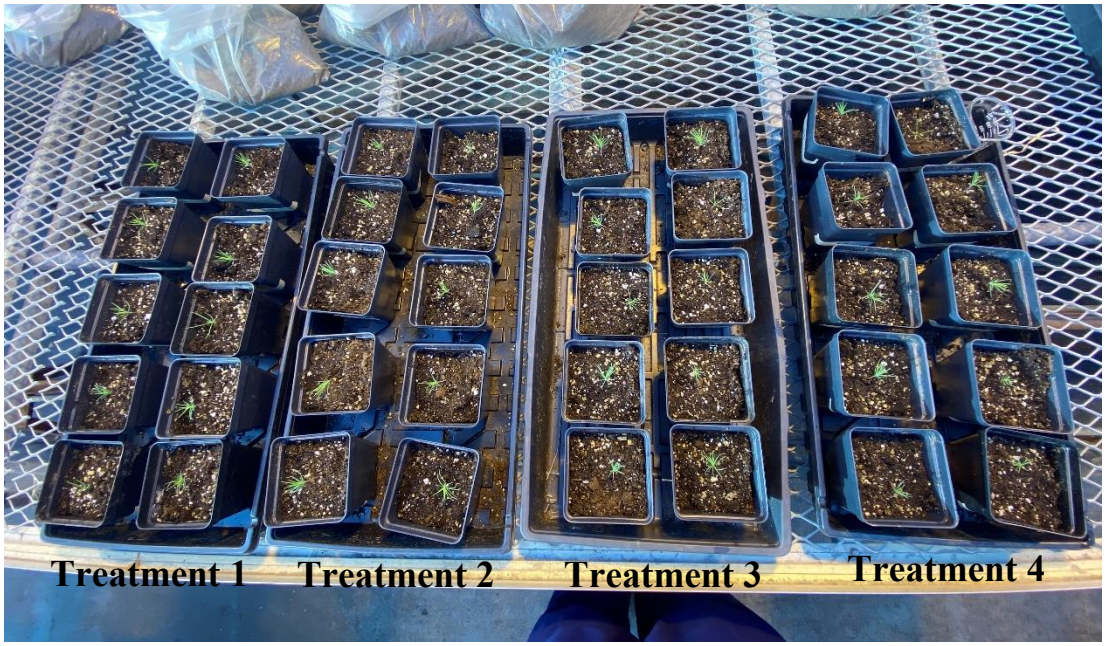

Figure 3. The set-up of the 4 treatments in the greenhouse at Lakehead University

Jack pine was chosen for this study because they are a member of Pinaceae which is one of the main families that ectomycorrhizal fungi form associations with (Toju and Sato 2018). When ectomycorrhizal fungal associations form on the roots of species in the genus *Pinus* they appear as short, dichotomously branched roots (W. H. P. 1943). This means that when counting associations during data collection the data will be more accurate as the associations will be easier to see.

Research Conduction

The research conduction started on November 17th, 2022, and occurred in the Lakehead University greenhouse. On November 17th, all treatments received 100 mL of water. The date that each treatment received water can be seen in the Appendices. At each watering, the pots within the scheduled treatments received 50 mL of water each. The water was measured with a beaker.

Data Collection

The trees received their final watering on February 20th, 2023, and grew for approximately 12 weeks. The 40 seedlings were brought from the greenhouse to the mycology lab at Lakehead University. The data collection took place over 4 days (February 21st-24th, 2023) with one treatment group being harvested and examined each day. The first thing done when working with a seedling was to dump the soil out of the pot then carefully shake all soil from the roots. The roots were then run under the tap to remove as much soil as possible before being transferred to large Petri dish filled with water. The dish was placed under a dissecting microscope and any remaining large clumps of soil were removed using two needles. The roots of the seedlings were untangled, and the number of short roots were counted and marked down using a tally system. A photo of short roots under the dissecting scope can be seen below in Figure 4 (b). The same methods were repeated for all seedlings in treatment groups 3 and 4. Treatment groups 1 and 2 underwent the same treatment but while they were in the Petri dish their mycorrhizal associations were counted as well. An example of a mycorrhizal association can be seen below in Figure 4 (a).

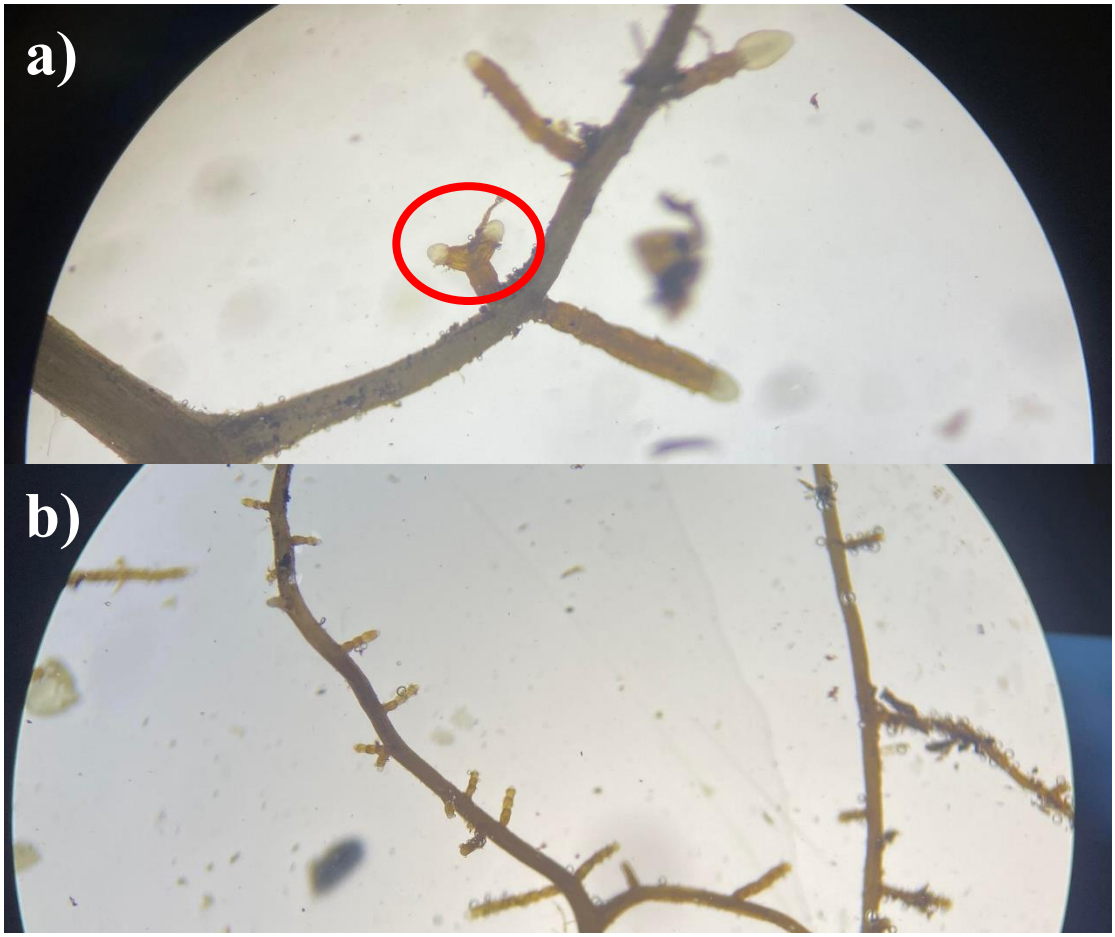

Figure 4 (a). An ectomycorrhizal association (circled in red) on a short root of a jack pine seedling, seen under the dissecting microscope; (b) Short roots of a jack pine seedling, seen under the dissecting microscope.

After the roots were counted the seedling was placed in between two paper towels to keep the roots moist. In between every five trees, the trees were placed on a board and a photo was taken. The photos all of seedlings can be seen in the Appendices. The seedlings were then placed into labelled paper bags. After 4 days of data collection all of the seedlings were in paper bags. The labelled paper bags were then placed into the drier in the mycology lab. Following drying for 3 days at 95 (°C), the seedlings were weighed on a balance to the nearest milligram. The seedlings were removed from the paper bags

with forceps and then placed on the balance. The dry weight was recorded and the process was repeated for all 39 seedlings.

Data Analysis

The data was recorded in a Microsoft Excel spreadsheet while it was being collected. Once collection of data was complete the percentage of mycorrhizal root colonization within treatment groups 1 and 2 were calculated. SPSS was used to determine the significance of the data. To do this the data was coded in Microsoft Excel and then the files were transported to SPSS. Two way ANOVAs were conducted in SPSS for dry weight and for short root count using water and inoculate as the two factors (independent variables). One-way ANOVAs were conducted in SPSS for amount of mycorrhizal associations and colonization percentage using water as the independent variable.

RESULTS

Only 39 seedlings survived to be collected for data, seedling 1-1 died. Results were collected using Microsoft Excel and SPSS Statistics. The significance value used in this study was $P = 0.1$. A table of raw data can be seen in the Appendices.

Dry Weight

The dry weight of all treatments were measured on a balance to the nearest milligram. The mean dry weight for all four treatment groups can be seen below in Table 2.

Table 2. Mean dry weights of the four treatment groups

Treatment Group	Dry Weight Average (grams)
Treatment 1 (Watered twice a week and inoculated)	0.082
Treatment 2 (Watered once a week and inoculated)	0.072
Treatment 3 (Watered twice a week and not inoculated)	0.078
Treatment 4 (Watered once a week and not inoculated)	0.061

As seen above in Table 2, treatment 1 had the highest dry weight average of 0.082 grams. The lowest average dry weight was found in treatment 4 at 0.061 grams. A bar graph depicting the average dry weight of all four treatments can be seen below in Figure 5.

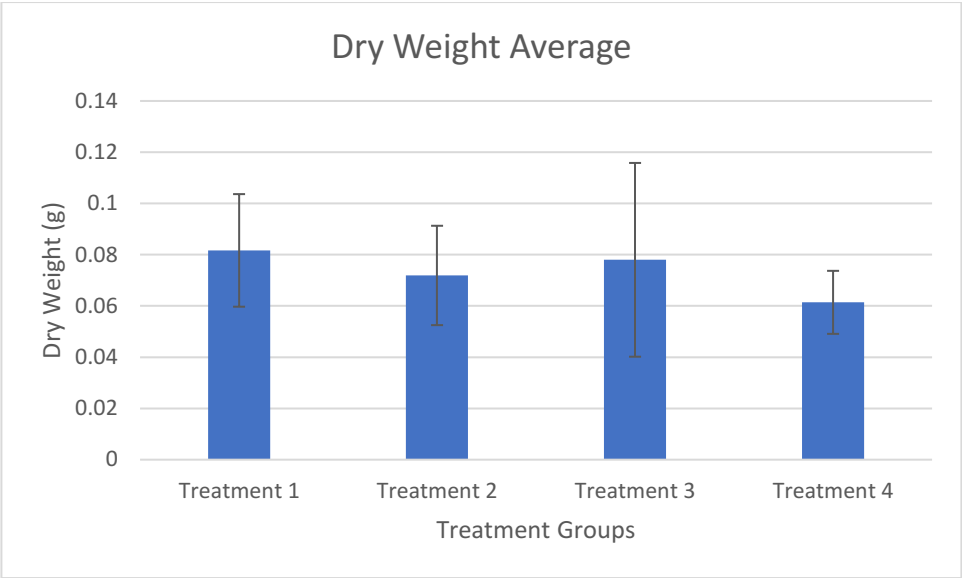

Figure 5. Average dry weights of the four treatments groups and bars depicting the standard deviation for each average.

A two way ANOVA was run in SPSS on the dry weights of all 39 seedlings. The two independent variables in the ANOVA were the amount of waterings the seedlings had received (once or twice per week) and if the seedling had been inoculated or not. The dependent variable was the dry weight of the seedlings. The difference in dry weights between the inoculated and non-inoculated seedlings was insignificant. The significance value derived from the two way ANOVA was 0.379 which is considerably higher than the significance value of the study (0.1). The difference in dry weights between the seedlings watered once and the seedlings watered twice was insignificant. The significance value taken from the two way ANOVA was 0.106 which is only slightly higher than the significance value for this study (0.1). Below in Figure 6 a graph depicts the difference between the dry weights between the seedlings watered once versus the seedlings watered twice.

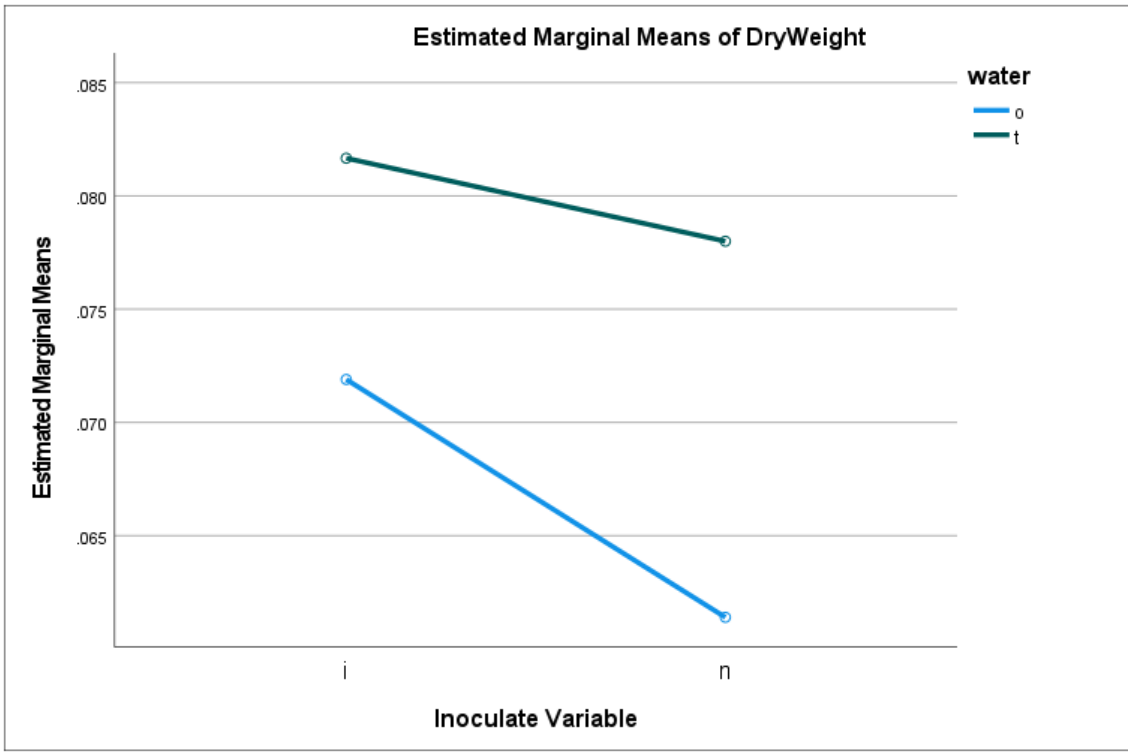

Figure 6. Graph depicting the marginal means of the dry weight between the seedlings watered once (o) and the seedlings watered twice (t).

Short Root Count

The average short root counts for the four treatment types can be seen depicted below in Table 3.

Table 3. Average short root counts for all four treatment types.

Treatment Group	Short Root Count Average
Treatment 1 (Watered twice a week and inoculated)	207
Treatment 2 (Watered once a week and inoculated)	213

Treatment 3 (Watered twice a week and not inoculated)	204
Treatment 4 (Watered twice a week and not inoculated)	194

As seen above in Table 3 the treatment type with the highest short root count average was treatment 2 with an average of 213 short roots per seedling. The treatment with the lowest average short root count was treatment 4 with an average of 194 short roots per seedling. A bar graph depicting the average short root counts for each treatment group can be seen below in Figure 7.

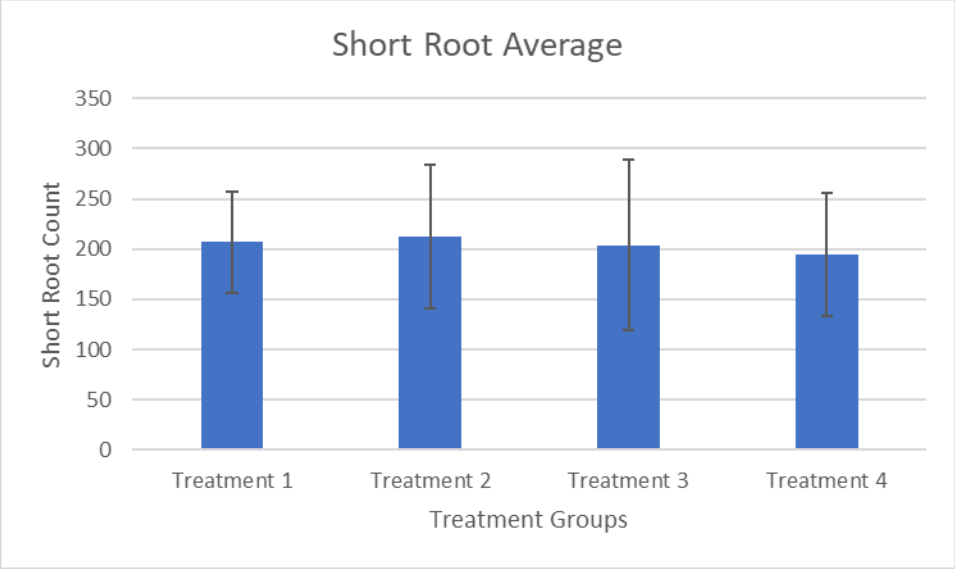

Figure 7. Average short root counts of the four treatments groups and bars depicting the standard deviation

A two way ANOVA was run in SPSS Statistics on the short root counts of all 39 seedlings. The two independent variables were the number of waterings the seedlings

had received per week (once and twice) and if the seedling had been inoculated with ectomycorrhizal fungi or not. The results from the two way ANOVA indicated that there was not a significant difference between the short root count of the seedlings watered once versus the seedlings watered twice. The significance value derived from the two way ANOVA was 0.930 which is greater than the significance value of the study (0.1). The difference between the average short roots of the inoculated and the non inoculated fungi was insignificant. The significance value taken from the two way ANOVA was 0.628 which is greater than the significance value of this study (0.1).

Mycorrhizal Associations

The number of mycorrhizal associations was counted using a dissecting microscope. The average mycorrhizal associations for the two inoculated treatment groups (1 and 2) can be seen below in Table 4.

Table 4. Average mycorrhizal associations per seedling in inoculated treatment groups

Treatment Group	Mean Mycorrhizal Associations
Treatment 1 (Watered twice a week and inoculated)	11
Treatment 2 (Watered once a week and inoculated)	12
Treatment 3 (Watered twice a week and not inoculated)	0
Treatment 4 (Watered once a week and not inoculated)	0

Above in Table 4 it is seen that there is limited difference between the average mycorrhizal associations per seedling between the seedlings that were watered twice a week and inoculated (Treatment 1) versus seedlings that were watered once a week and inoculated (Treatment 2). A one way ANOVA test was run on the mycorrhizal association count of the 19 inoculated seedlings. The difference between the mycorrhizal associations in the seedlings watered once versus the seedlings watered twice was insignificant. The significance value derived from the ANOVA test was 0.854 which is higher than the significance value for this study (0.1). In Table 4 it is also seen that no mycorrhizal associations were observed on the non-inoculated seedlings.

A one-way ANOVA test was also run to compare the difference in the amount of mycorrhizal associations between the inoculated and non-inoculated seedlings. It was found that there was a significance difference between the mycorrhizal associations in the inoculated and non-inoculated treatment groups. The difference was 0.001 which is considerably lower than the significance value of this study (0.1). Below in Figure 8 a graph depicts the difference in mycorrhizal associations between the non-inoculated and inoculated treatment groups.

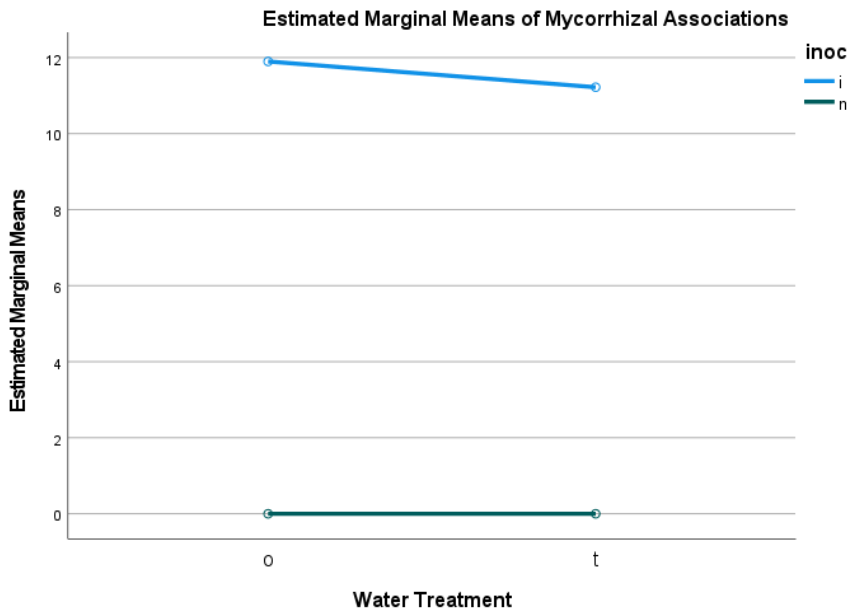

Figure 8. Graph depicting the marginal means of the mycorrhizal associations between the seedlings inoculated (i) and the seedlings non-inoculated (n).

Colonization of Short Roots by Ectomycorrhizal Fungi

The percentage of short roots colonized by mycorrhizal fungi was calculated by dividing the total number of short roots by the number of mycorrhizal associations found in the seedling. It was calculated for the inoculated treatment groups (treatments 1 and 2). The average colonization percentage for treatment groups one and two can be seen below in Table 5.

Table 5. Average percentage of short roots colonized by mycorrhizal fungi

Treatment Group	Average Colonization (%)
Treatment 1	5.63
Treatment 2	5.97

As seen above in Table 5 the average percentage of short root colonization by ectomycorrhizal fungi is slightly higher in treatment two than in treatment one with the average colonization being 5.97, and 5.63 percent respectively. A one way ANOVA test was run on the calculated colonization of the 19 inoculated seedlings in SPSS Statistics. The number of times the seedlings were watered throughout the week (once or twice) was used as the independent variable with the colonization (%) being the dependent variable. The results of the ANOVA indicated that the difference in the short root colonization between the seedlings watered once a week versus the seedlings watered twice a week was insignificant. The significance value taken from the ANOVA test was 0.854 which is higher than the significance value of this study (0.1). There was no colonization calculated for the non-inoculated seedlings because there were no mycorrhizal associations formed.

DISCUSSION

Ectomycorrhizal fungi form associations with many of the dominant plant families in today's forests such as Fagaceae, Pinaceae, Betulaceae and Dipterocarpaceae (Toju and Sato 2018). The benefits that these fungi provide to the tree species that they are in association with promote those families dominance in current forests (Toju and Sato 2018). The benefits provided to the tree from the fungi include protection from toxic metals, uptake of nutrients, and increased access to water (Stuart and Plett 2020, Policelli *et al*. 2020, Colpaert *et al*. 2011). Studies suggest that ectomycorrhizal fungus associations had a significant positive effect on seedlings under drought-stress (Dixon *et al*. 1980, Park *et al.* 1983, Arya *et al*. 2021). In this study no significant results were found but differences in data were observed.

The first hypothesis of this study was the jack pine seedlings that have been inoculated with *Hebeloma longicaudum* will exhibit higher levels of growth and greater number of colonized short roots than those seedlings not inoculated. This hypothesis is partly proven as the jack pine seedlings that had been inoculated with the ectomycorrhizal fungus did show a significantly greater numbers of colonized short roots than the jack pine seedlings that had not been inoculated. The reason for the significant difference is that there were no mycorrhizal associations observed on any of the seedlings without the inoculum.

The second hypothesis was that the jack pine seedlings inoculated with *Hebeloma longicaudum*. and subjected to a frequent watering regime will show greater amounts of growth compared with those seedlings that were not inoculated and subjected to a drought-like watering regime. This hypothesis was disproven in this study as there were

no significance differences observed in growth (dry weight) between the inoculated and frequently watered seedlings and the seedlings that were not inoculated and subjected to a drought-like watering regime.

The species *Hebeloma longicaudum* was chosen for this study. The genus *Hebeloma*. belongs to the family Cortinariaceae and most members of the genus are ectomycorrhizae formers (Marmeisse *et al*. 1999). Members of this genus are found in Europe, Asia, North America, and Australia on a wide range of hosts (Marmeisse *et al*. 1999). *Hebeloma* spp. are early stage colonizers and can form associations with seedlings and young trees efficiently (Marmeisse *et al*. 1999). The species *Hebeloma longicaudum* is an ectomycorrhizal fungus that forms associations with pine and spruce species (Wichlacz *et al*. 1999). This fungus has potential for successful large-scale nursery inoculation of conifer seedlings (Wichlacz *et al*. 1999) which is a primary reason why it was chosen for this study.

The dry weights of the seedlings were one of the variables of interest in this study. Treatment one had the highest dry weight average of all treatments with the average being 0.082 grams. This was expected as this treatment was watered twice a week and inoculated with ectomycorrhizal fungus. A study done by Parke *et al*. (1983) found that inoculated Douglas-fir seedlings exhibited higher dry weight than non inoculated seedlings. Aryal *et al*. (2021) found that ectomycorrhizal fungi colonization on the roots of chestnut seedlings increased seedling above ground biomass in the greenhouse.

A two-way ANOVA test was run to compare the dry weights of the seedlings between all four treatment groups. The difference in dry weights was found to be insignificant with a value of 0.379. The most likely reason why this result is insignificant is that the

mycorrhizal associations were not given enough time to develop fully before data collection. The two-way ANOVA results showed that the difference in dry weights between the seedlings subjected to a drought-like watering regime and a normal watering regime was not significant. The significance value for this difference was 0.106 which is only slightly higher than the significance value for this study (0.1). This compares with results found in a study done by Sultana *et al*. (2021) where a significant reduction was found in total dry biomass of seedlings under 25% soil water content versus 50% soil water content.

The difference in short root count between the treatment groups was found to be insignificant by a two-way ANOVA run in SPSS Statistics. The treatment with the highest average count of short roots was treatment 2 with the average being 213 short roots per seedling. This disproves the second hypothesis of this study as treatment 2 was subjected to a drought-like watering regime.

A significant difference was noticed in the mycorrhizal associations between the inoculated and inoculated seedlings. The significance value derived from a two-way ANOVA for this difference was less than 0.01. The reason for this difference is that no mycorrhizal associations were noticed on the non-inoculated seedlings. Parke *et* al. (1983) also observed no mycorrhizal associations on their non-inoculated Douglas fir seedlings.

The colonization percentage of seedling short roots was only calculated for treatments 1 and 2. This is because there was no value for the number of mycorrhizal associations on treatment groups 3 and 4 (not inoculated) so no calculation could be completed. Treatment 2 had the higher colonization percentage even though it was receiving less

water than treatment 1. The lack of difference between the two treatment groups is most likely due to *Hebeloma longicaudum* not having enough time to develop associations.

Errors

In this experiment, few significant results were observed. Factors that may have contributed to this result are discussed in the following paragraphs.

The jack pine seeds were all germinated on the same date in the greenhouse but when the seedlings were transplanted not all 40 seedlings demonstrated an equal amount of root and shoot growth. Tree 1 in treatment group one died three weeks into the research conduction. The lack of sameness when the seedlings were inoculated could have further skewed the results of this study.

The seedlings were grown in the main Lakehead University greenhouse which is not a temperature controlled growing area. The temperature in the greenhouse varied day to day depending on the outside temperature and the amount of sunlight during the day. Variation of temperature caused the amount of soil moisture to vary in between treatments which could have affected the results. To counteract this the trays of pots and the individual pots were moved around at random.

The lack of significance in the mycorrhizal root associations could be explained by not allowing the fungi enough time to colonize the root systems of the seedlings. The seedlings in this study were only grown for four months with ectomycorrhizal fungi while a study done by Parke *et al* (1983) allowed the seedlings and mycorrhizal fungi to grow together for 7 months before data collection.

If this study was to be replicated, it is recommended that soil water content (%) be used to control the amount of water the seedlings receive each week as opposed to a set amount of water. This will keep soil water conditions more consistent and provide for more accurate data.

Hebeloma longicaudum was the ectomycorrhizal fungus chosen for this study. If this study was repeated then a different species, or multiple species of ectomycorrhizal fungi would be used. Multiple species being used in the study would provide more data and give more accurate results. The ectomycorrhizal fungi would also be given more time to form associations. This will make it clear whether lack of mycorrhizal associations is due to the conditions of the study or too little time.

CONCLUSION

Despite lack of significant results in this study, copious amounts of research have shown that ectomycorrhizal fungus associations mitigate the effects of drought on tree seedlings. The results from this study show how drought has an effect on seedling growth. This should be taken into account when looking for solutions to mitigate the effects of climate change induced drought on Canadas forest and forestry industry.

LITERATURE CITED

Adams, H. D., and T. E. Kolb. 2005. Tree growth response to drought and temperature in a mountain landscape in northern Arizona, USA. Journal of Biogeography. 32(9): 1629-1640. https://doi.org/10.1111/j.1365-2699.2005.01292.x

Adams, R. M. 1989. Global Climate Change and Agriculture: An Economic Perspective. American Journal of Agriculture Economics. 71(5):1272 – 1279. https://doi.org/10.2307/1243120.

Al-Kaisi, M. 2017. How Drought Affects Soil Health. Integrated Crop Management News. Iowa State University Extension and Outreach.

Allen, C. D., A. K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D. D. Breshears, E. H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J-H. Lim, G. Allard, S. W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management. 259(4): 660-684. https://doi.org/10.1016/j.foreco.2009.09.001

Aryal, P., S. J. Meiners, and B. S. Carlsward. 2021. Ectomycorhizae determine chestnut seedling growth and drought response. Agroforestry Systems. 95: 1251-1260. https://doi.org/10.1007/s10457-020-00488-4

Aucina, A., M. Rudawska, T. Leski, A. Skridaila, E. Riepsas, and M. Iwanski. 2007. Growth and Mycorrhizal Community Structure of *Pinus sylvestris* Seedlings following the Addition of Forest Litter. Applied and Environmental Microbiology. 73(15): 4867 – 4973. https://doi.org/10.1128/AEM.00584-07.

Averill, C., J. M. Bhatnagar, M. C. Dietze, W. D. Pearse, and S. N. Kivlin. 2019. Global imprint of mycorrhizal fungi on whole-plant nutrient economics. Biological Sciences. 1116(46): 23163 – 23168. https://doi.org/10.1073/pnas.1906655116.

Barnes, A. 2019. 2018 Economic State of the B.C. Forest Sector. Economic Services Branch.

Brecka, A. F. J., C. Shai, H. Y. H. Chen. 2018. Climate change impacts on boreal forest timber supply. Forest Policy and Economics. 92: 11-21. https://doi.org/10.1016/j.forpol.2018.03.010.

Buscardo, E., R. C. Souza, P. Meir, J. Geml, S. K. Schmidt, A. C. L. da Costa, and L. Nagy. 2021. Effects of natural and experimental drought on soil fungi and biogeochemistry in an Amazon rain forest. Communications Earth and Environment. 2(55). https://doi.org/10.1038/s43247-021-00124-8.

Chodak, M., M. Golebiewski, J. Morawska-Ploskonka, K. Kuduk, and M. Niklinska. 2015. Soil chemical properties affect the reaction of forest soil bacteria to

drought and rewetting stress. Annals of Microbiology. 65: 1627 - 1637. https://doi.org/10.1007/s13213-014-1002-0.

Colpaert, J. V., J. H. L. Wevers, E. Krznaric, and K. Adriaensen. 2011. How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Annals of Forest Science. 68: 17-24. https://doi.org/10.1007/s13595-010-0003-9.

Cook, B. I., J. S. Mankin, and K. J. Achukaitis. 2018. Climate Change and Drought: From Past to Future. Current Climate Change Reports. 4: 164-179. https://doi.org/10.1007/s40641-018-0093-2.

Dighton, J. 2009. Mycorrhizae. p. 153 – 162. in: Schaechter, M (Ed). Encyclopedia of Microbiology (Third Edition). Elsevier, Inc. Amsterdam. https://www.sciencedirect.com/science/article/pii/B9780123739445003278.

Dixon, R. K., G. M. Wright, G. T. Behrns, R. O. Teskey, and T. M. Hinckley. 1980. Water deficits and root growth of ectomycorrhizal white oak seedlings. Canadian Journal of Forest Research. 10(4): 545-548. https://doi.org/10.1139/x80-089.

Dobbertin, M. 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. European Journal of Forest Research. 124: 319 – 333. https://doi.org/10.1007/s10342-005-0085-3

Dong, Y., Z. Wang, H. Sun, W. Yang, and H. Xu. 2018. The response patterns of arbuscular mycorrhizal and ectomycorrhizal symbionts under elevated CO2: a meta-analysis. Frontiers in microbiology. 9(1248). https://doi.org/10.3389/fmicb.2018.01248.

Douglas, S. M. 2023. Drought Stress, Tree Health, and Management Implications. The Connecticut Agricultural Experiment Station.

Duddridge, J. A., A. Malibari, and D. J. Reed. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature. 287: 834 – 836. https://doi.org/10.1038/287834a0.

Frelich, L. E., R. A. Montgomery, and P. B. Reich. 2021. Seven Ways a Warming Climate Can Kill the Southern Boreal Forest. Impact of Climate Change on Biome Distributions in Forests. 12(5):560. https://doi.org/10.3390/f12050560.

Frank, H. E. R., and K. Garcia. 2021. Benefits provided by four ectomycorrhizal fungi to *Pinus taeda* under different external potassium availabilities. Mycorrhiza. 31: 755-766. https://doi.org/10.1007/s00572-021-01048-z.

Gauthier, S., P. Bernier, T. Kuulavinen, A. Z. Shvidenko, and D. G. Schepaschenko. 2015. Boreal forest health and global change. Science. 349(6250): 819-822.

Government of British Columbia. 2017. FACTSHEET: Reforestation in B.C. Ministry of Forests, Lands and Natural Resource Operations. Found at:

https://news.gov.bc.ca/factsheets/factsheet-reforestation-in-bc. Accessed on: December 15th, 2022.

Government of Canada. 2021. Boreal forest. Natural Resources Canada. Natural Resources Canada. Found at: https://www.nrcan.gc.ca/our-natural-resources/forests/sustainable-forest-management/boreal-forest/13071. Accessed on: October 24th, 2022.

Government of Canada. 2020. 8 facts about Canada's boreal forest. Natural Resources Canada. Found at: https://www.nrcan.gc.ca/our-natural-resources/forests/sustainable-forest-management/boreal-forest/8-facts-about-canadas-boreal-forest/17394. Accessed on: October 24th, 2022.

Kays, I., and A. Malik. 2020. Boreal Forests: Distributions, Biodiversity, and Management. Life on Land. Encyclopedia of the UN Sustainable Development Goals. pp. 1-12. https://doi.org/10.1007/978-3-319-.

Kranabetter, J. M., D. M. Durall, and W. H. MacKenzie. 2009. Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. Mycorrhiza. 19: 99-111. https://doi.org/10.1007/s00572-008-0208-z.

Krupa. P., and J. Kozdroj. 2004. Accumulation of heavy metals by ectomycorrhizal fungi colonizing birch trees growing in an industrial desert soil. World Journal of Microbiology and Biotechnology. 20: 427-430. https://doi.org/10.1023/B:WIBI.0000033067.64061.f3.

Lindsey, R., and L. Dahlman. 2022. Climate Change: Global Temperature. NOAA. Found at: https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature. Accessed on: October 23rd, 2022.

Lehto, T. and J. J. Zwiazek. 2011. Ectomycorrhizas and water relations of trees: a review. Mycorrhiza. 21: 71-90. https://doi.org/10.1007/s00572-010-0348-9.

Luyssaert, S., E. D. Schulze, A. Borner, A. Law, P. Ciais, and J. Grace. 2008. Old-growth forests as global carbon sinks. Nature. 455: 213-215. https://doi.org/10.1038/nature07276.

Marmeisse, R., H. Gryter, P. Jargeat, L. Frassinet-Tachet, G. Gay, and J. C. Debaud 1999. *Hebeloma*. Pp 89-127 *in* Cairney J. W. G., and S. M. Chambers (Eds.) Ectomycorrhizal Fungi: Key Genera in Profile. Spring-Verlag, Berlin.

Mendivelso, H. A., J. J. Camarero, E. Gutierrez, and P. A. Zuidema. 2014. Time-dependent effects of climate and drought on tree growth in a Neotropical dry forest: Short-term tolerance vs. long-term sensitivity. Agricultural and Forest Meteorology. 188:13-23. https://doi.org/10.1016/j.agrformet.2013.12.010.

Moore, J. A. M., J. Jiang, W. M. Post, and A. T. Classen. 2015. Decomposition by ectomycorrhizal fungi alters soil carbon storage in a simulation model. Ecosphere. 6(3): 1-16. https://doi.org/10.1890/ES14-00301.1.

Ortega, U., M. Dunabeitia, S. Menendez, C. Gonzalez-Murua and J. Majada. 2004. Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of *Pinus radiata* in different water regimes. Tree Physiology. 24(1): 65-73. https://doi.org/10.1093/treephys/24.1.65.

Overstreet, R. M., and J. M. Lotz. 2016. Host–symbiont relationships: understanding the change from guest to pest. The Rasputin effect: when commensals and symbionts become parasitic. Advances in Environmental Microbiology. 3: 27-64. https://doi.org/10.1007/978-3-319-28170-4_2.

Park, J. L., R. G. Linderman, and C. H. Black. 1983. The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. The New Phytologist. 95(1): 83-95. http://www.jstor.org/stable/2434174.

Pena, R., and A. Polle. 2013. Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. The ISME Journal. 8: 321-330. https://doi.org/10.1038/ismej.2013.158

Policelli, N., T. R. Horton, A. T. Hudon, T. R. Patterson and J. M. Bhatnagar. 2020. Back to roots: the role of ectomycorrhizal fungi in boreal and temperate forest restoration. Frontiers in Forests and Global Change. 3(97): 1-15. https://doi.org/10.3389/ffgc.2020.00097

Price, D. T., R. I. Alfaro, K. J. Brown, M. D. Flannigan, R. A. Fleming, E. H. Hogg, M. P. Girardin, T. Lakusta, M. Johnston, D. W. McKenney, J. H. Pedlar, T. Stratton, R. N. Sturrock, I. D. Thompson, J. A. Trofymow, and L. A. Venier. 2013. Anticipating the consequences of climate change for Canada's boreal forest ecosystems. Environmental Reviews. 21(4): 322-365. https://doi.org/10.1139/er-2013-0042

Qu, L., K. Makoto, D. Choi, A. M. Quoreshi, and T. Koike. 2010. The Role of Ectomycorrhiza in Boreal Forest Ecosystem. In: Osawa, A., O. Zyryanova, Y. Matsuura, T. Kajimoto, R. Wein (Eds). Permafrost Ecosystems. Ecological Studies Vol. 209. Springer, Dordrecht, Germany. pp. 413-425. https://doi.org/10.1007/978-1-4020-9693-8_21.

Sanchez-Pinillos, M., L. D'Orangeville, Y. Boulanger, P. Comeau, J. Wang, A. R. Taylor, and D. Kneeshaw. 2021. Sequential droughts: A silent trigger of boreal forest mortality. Global Change Biology. 28(2):542-556. https://doi.org/10.111/gcb.15913.

Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H. Huang, N. Harnik, A. Leetmaa, N. Lau, C. Li, J. Velez, and N. Naik. 2007. Model projections of an

imminent transition to a more arid climate in southwestern North America. Science. 316(5828): 1181-1184. https://doi.org/10.1126/science.1139601.

Senf, C., A. Buras, C. S. Zang, A. Rammig, and R. Seidl. 2020. Excess forest mortality is consistently linked to drought across Europe. Nature Communications. 11(6200). https://doi.org/10.1038/s4146qqq7-020-19924-1.

Siddiqui, Z. A., and J. Pichtel. 2008. Mycorrhizae: An Overview. p 1-35. In: Siddiqui, Z. A., S. A. Mohd, and Kazuyashi, F. (Eds.) Mycorrhizae: Sustainable Agriculture and Forestry. Springer Dordrecht. 362 pp. https://doi.org/10.1007/978-1-4020-8770-7.

Stuart, E. K., and K. L. Plett. 2020. Digging Deeper: In Search of the Mechanisms of Carbon and Nitrogen Exchange in Ectomycorrhizal Symbioses. Frontiers in Plant Science. 10(1658). https://doi.org/10.3389/fpls.2019.01658.

Sultana, N., S. H. Limon, M. S. Rahman, A. Akther, S. Salekin, D. F. Meason, and M. Bloomberg. 2021. Germination and growth responses to water stress of three agroforestry tree species from Bangladesh. Environmental Challenges. 5(100256). https://doi.org/10.1016/j.envc.2021.100256.

Swaty, R. L., R. J. Deckert, T.G. Whitham, and C. A. Gehring. 2004. Ectomycorrhizal abundance and community composition shifts with drought: predictions from tree rings. Ecological Society of America. 85(4): 1072-1084. https://doi.org/10.1890/03-0224.

Tam, P. C. F. 1995. Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. Mycorrhiza. 5: 181-187. https://doi.org/10.1007/BF00203335.

Toju, H., and H. Sato. 2018. Root-associated fungi shared between arbuscular mycorrhizal and ectomycorrhizal conifers in a temperate forest. Frontiers in Microbiology. 9(433). https://doi.org/10.3389/fmicb.2018.00433.

Trenberth, K. E. 2013. Changes in precipitation with climate change. Climate Research. 47: 123-138. https://doi.org/10.3354/cr00953

United States Environmental Protection Agency. 2022. Climate Change Indicators: Atmospheric Concentrations of Greenhouse Gases. Found at: https://www.epa.gov/climate-indicators/climate-change-indicators-atmospheric-concentrations-greenhouse-gases. Accessed on: November 1st, 2022.

Van der Heijden, M. G. A., F. M. Martin, M. Selosse, and I. R. Sanders. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist. 205(4): 1406 – 1423. https://doi.org/10.1111/nph.13288.

Westfall, J., and M. Duthie-Holt. 2021. Summary of Forest Health Conditions in British Columbia. Ministry of Forests, Lands, Natural Resource Operations and Rural Development.

Whitehead, D. 2011. Forests as carbon sinks—benefits and consequences. Tree Physiology. 31(9). 893-902. https://doi.org/10.1093/treephys/tpr063.

W. H. P. 1943. Review: The Mycorrhizal Relations of Pinus. Journal of Ecology. 31(1): 41-44. https://www.jstor.org/stable/2256786.

Wichlacz, M., W. A. Ayer, L. S. Trifonov, P. Chakravarty, and D. Khasa. 1999. *cis*-Fused caryophyllenes from liquid cultures of *Hebeloma longicaudum*. Phytochemistry. 51(7): 873-877. https://doi.org/10.1016/S0031-9422(99)00117-X.

Worley, J. F., and Hacskaylo E. 1959. The effect of available soil moisture on the mycorrhizal association of Virginia Pine. Forest Science. 5(3): 267-268. https://doi.org/10.1093/forestscience/5.3.267.

APPENDICES

APPENDIX I

Table 6. Table depicting the dates that each treatment receives water

DATE	Treatment-1	Treatment-2	Treatment-3	Treatment-4
2022-11-17	X	X	X	X
2022-11-21	X		X	
2022-11-24	X	X	X	X
2022-11-28	X		X	
2022-12-01	X	X	X	X
2022-12-05	X		X	
2022-12-08	X	X	X	X
2022-12-12	X		X	
2022-12-15	X	X	X	X
2022-12-19	X		X	
2022-12-22	X	X	X	X

2022-12-26	X		X	
2022-12-29	X	X	X	X
2023-01-02	X		X	
2023-01-05	X	X	X	X
2023-01-09	X		X	
2023-01-12	X	X	X	X
2023-01-16	X		X	
2023-01-19	X	X	X	X
2023-01-23	X		X	
2023-01-26	X	X	X	X
2023-01-30	X		X	
2023-02-02	X	X	X	X
2023-02-06	X		X	
2023-02-09	X	X	X	X
2023-02-13	X		X	
2023-02-16	X	X	X	X
2023-02-20	X		X	
X=treatment receives 50 mL of water				

APPENDIX II

Table 7. Raw data collected

Tree Number	Mycorrhizal Associations	Dry Weight	Number of Mycorrhizal Associations	Count of Short Roots	Colonization (%)
1~1	YES	x	x	x	x
1~2	YES	0.086	5	215	2.325581395
1~3	YES	0.083	8	234	3.418803419
1~4	YES	0.095	11	277	3.971119134
1~5	YES	0.125	15	258	5.813953488
1~6	YES	0.095	13	236	5.508474576
1~7	YES	0.061	18	186	9.677419355
1~8	YES	0.059	14	179	7.82122905
1~9	YES	0.057	7	120	5.833333333
1~10	YES	0.074	10	158	6.329113924
2~1	YES	0.076	12	369	3.25203252
2~2	YES	0.064	18	182	9.89010989
2~3	YES	0.081	37	223	16.59192825
2~4	YES	0.104	7	267	2.621722846
2~5	YES	0.079	4	195	2.051282051
2~6	YES	0.096	7	262	2.671755725
2~7	YES	0.065	5	188	2.659574468
2~8	YES	0.041	6	126	4.761904762
2~9	YES	0.063	6	168	3.571428571
2~10	YES	0.05	17	146	11.64383562
3~1	NO	0.093	X	292	X
3~2	NO	0.116	X	249	X

3~3	NO	0.045	X	123	X
3~4	NO	0.055	X	173	X
3~5	NO	0.071	X	313	X
3~6	NO	0.099	X	192	X
3~7	NO	0.072	X	178	X
3~8	NO	0.037	X	100	X
3~9	NO	0.038	X	99	X
3~10	NO	0.154	X	319	X
4~1	NO	0.055	X	226	X
4~2	NO	0.057	X	131	X
4~3	NO	0.076	X	231	X
4~4	NO	0.074	X	308	X
4~5	NO	0.058	X	229	X
4~6	NO	0.071	X	235	X
4~7	NO	0.047	X	129	X
4~8	NO	0.074	X	175	X
4~9	NO	0.062	X	157	X
4~10	NO	0.04	X	122	X

APPENDIX III

Photos showing the seedlings in each treatment group

Figure 9. Seedlings 2-5 in treatment one (frequent watering regime and inoculated with fungus)

Figure 10. Seedlings 6-10 in treatment one (frequent watering regime and inoculated with fungus)

Figure 11. Seedlings 1-5 in treatment two (drought-like watering regime and inoculated with fungus)

Figure 12. Seedlings 6-10 in treatment two (drought-like watering regime and inoculated with fungus)

Figure 13. Seedlings 1-5 in treatment three (frequent watering regime and not inoculated)

Figure 14. Seedlings 6-10 in treatment three (frequent watering regime and not inoculated)

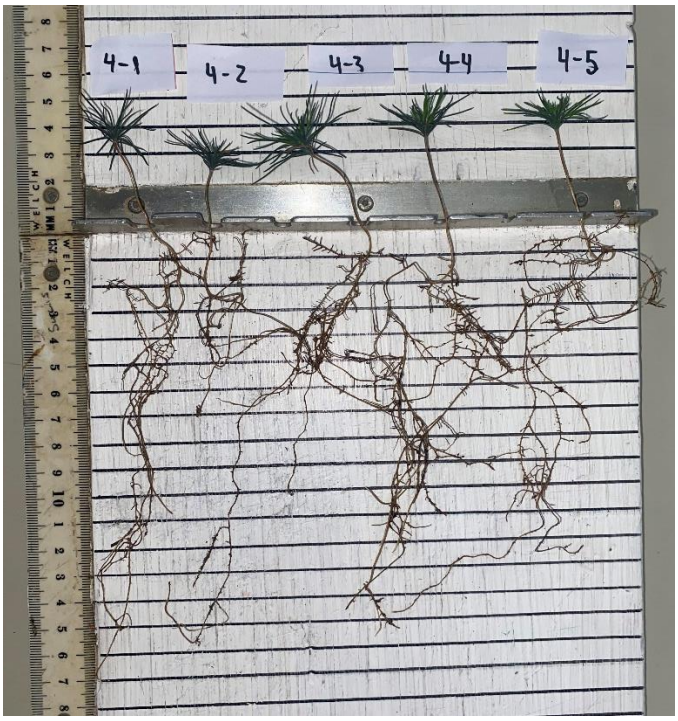

Figure 15. Seedlings 1-5 in treatment four (drought-like watering regime and not inoculated)

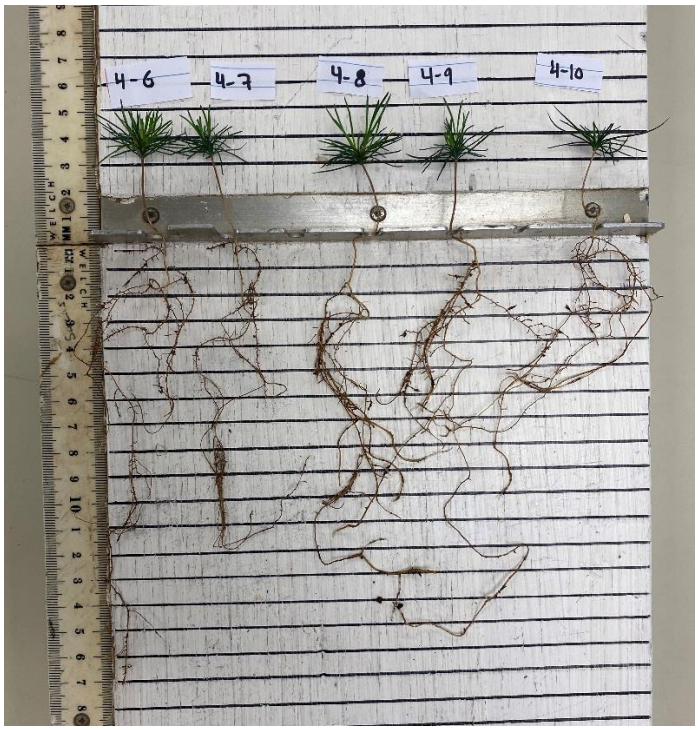

Figure 16. Seedlings 6-10 in treatment four (drought-like watering regime and not inoculated)