Propagation Methods and the Effectiveness of Fungal Inoculation on *Vaccinium* Species Native to Central British Columbia

Irene McKechnie

B.Sc., University of Northern British Columbia, 2003

Thesis Submitted in Partial Fulfillment of

The Requirements for the Degree of

Master of Science

In

Natural Resources and Environmental Studies

(Biology)

The University of Northern British Columbia

June 2009

Your file Votre r6f6rence
ISBN: 978-0-494-60824-1
Our file Notre r6f6rence
ISBN: 978-0-494-60824-1

ABSTRACT

There is great demand for the inclusion of berry-producing plants, such as *Vaccinium* species, in forest restoration and post-industrial reclamation efforts, due to their value to wildlife and traditional users of the land; however, the biology and propagation requirements of northern *Vaccinium* species and their potential for use in reclamation, restoration, and horticulture is largely unexplored. Propagation trials of *V. membranaceum, V. myrtilloides,* and *V. caespitosum* using seeds, rhizome cuttings, and hardwood cuttings were carried out to determine propagation protocols. Outdoor trials tested the influences of mycorrhizal inoculation and soil amendment on seedling survival. Seeds and rhizome cuttings were found to be the most effective propagation techniques for *Vaccinium.* Soil amendment increased outdoor seedling survival; however, none of the seedlings showed mycorrhizal colonization, regardless of inoculation treatment. Further studies of the effect of seedling age and soil characteristics on mycorrhizal colonization are required.

TABLE OF CONTENTS

List of Tables

List of Figures

Acknowledgement

Throughout this project I have received help and support from many people. Firstly, a large thank you to my co-supervisors, Hugues Massicotte and Philip Burton, for their guidance and support, as well as to Paul Sanborn for agreeing to round out my committee. I likely would never have started on this path without the support and prodding of Barbara Rayment of Birch Creek Nursery. Thank you to John Orlowsky and Steve Storch for their invaluable assistance and advice on the technical aspects of shrub propagation. Laboratory assistance was provided by Monika Gorzelak, Linda Tackaberry, and Lesley Dampier. Many people either accompanied me while collecting berries, for companionship and protection from the bears, or collected berries for me. A big thank you to Jillian Johnston, Laura Ryser, Hugues Massicotte, Phil Burton, Linda Tackaberry, Roy Rea, Erin Clark, and Kyla Warren for their berry-picking help. Field site preparation was made easier thanks to the help of Chris Knudson and Steve Patton of the UNBC Facilities department and their skid steer loader, and Susan Robertson, Monika Gorzelak, and Hugues Massicotte, who helped with the collection and distribution of soil amendments. The outdoor seedlings needed watering every day, and I thank Eduardo Bittencourt, Robin Steenweg, Crystal McRae, Erin Clark, William Douglas, Craig McKechnie, Sjoukje McKechnie, and Neil McKechnie for their help with this tedious task. Of course no project ever gets accomplished without the moral support of the people around you. I would like to thank former and present office-mates Marcel Macullo, Robin Steenweg, Eduardo Bittencourt, Becky Cadsand, and Robin Urquhart, lab-mates Monika Gorzelak, Susan Robertson, Nancy-Anne Rose, and Sean Haughian, and fellow grad students Erin Clark, Kyla Warren, and Crystal McRae for being there through the good and not-so-good times. Last, but certainly not least, I would like to thank my family for their constant support despite the many miles between us.

Financial support for this project was provided through a UNBC Graduate Entrance Scholarship, a UNBC Master's Scholarship, and an NSERC PGS-M awarded to Irene McKechnie as well as an NSERC Discovery Grant awarded to Hugues Massicotte.

1.0 Introduction and Literature Review

Vaccinium species occur throughout the world in many different climatic regions. Within this single genus, there are nine sections *{Oxycoccoid.es, Vaccinium, Oxycoccus, Vitis-idaea, Polycodium, Myrtillus, Batodendron, Herpothamnus, Cyanococcus,* and *Pyxothamnus*) and approximately 26 species occurring within North America (Vander Kloet 1988). Many ericaceous plants, including some *Vaccinium* species, are difficult to propagate with regular success under nursery settings. When seedlings do thrive in the nursery, they often have very low survival rates when transplanted to outdoor conditions (Cole and Spildie 2006). Much of the research conducted is performed with the commercially important high-bush blueberry *(V. corymbosum* L.) and cranberry (V. *macrocarpon* Aiton) plants, whereas the behaviour of species native to British Columbia is less well understood. This review will focus on the current state of knowledge of *Vaccinium* ecology and propagation techniques, with special attention paid to the role of mycorrhizal fungi. Where information is available, reference will be made to black huckleberry (V. *membranaceum* Douglas ex. Torr.), velvet-leaf blueberry (V. *myrtilloides* Michx.), and dwarf huckleberry *(V. caespitosum* Michx.), three common *Vaccinium* species which can be found in North-Central B.C.

1.1 Vaccinium ecology

1.1.1 Distribution

Species in the genus *Vaccinium* have been found in North America, South America, Asia, Africa, Europe, and the Pacific region (Jacquemart 1996, Vander Kloet 1988). Within these regions, *Vaccinium* species can be found from sea level to alpine environments. *Vaccinium membranaceum* is predominantly found in western North America at latitudes between 32°N and 60°N (Figure 1). It has been reported north to the Northwest Territories,

south to Arizona, west to Vancouver Island, and east to Ontario and Michigan (Barclay-Estrup 1987, Small and Catling 2005, Vander Kloet 1988).

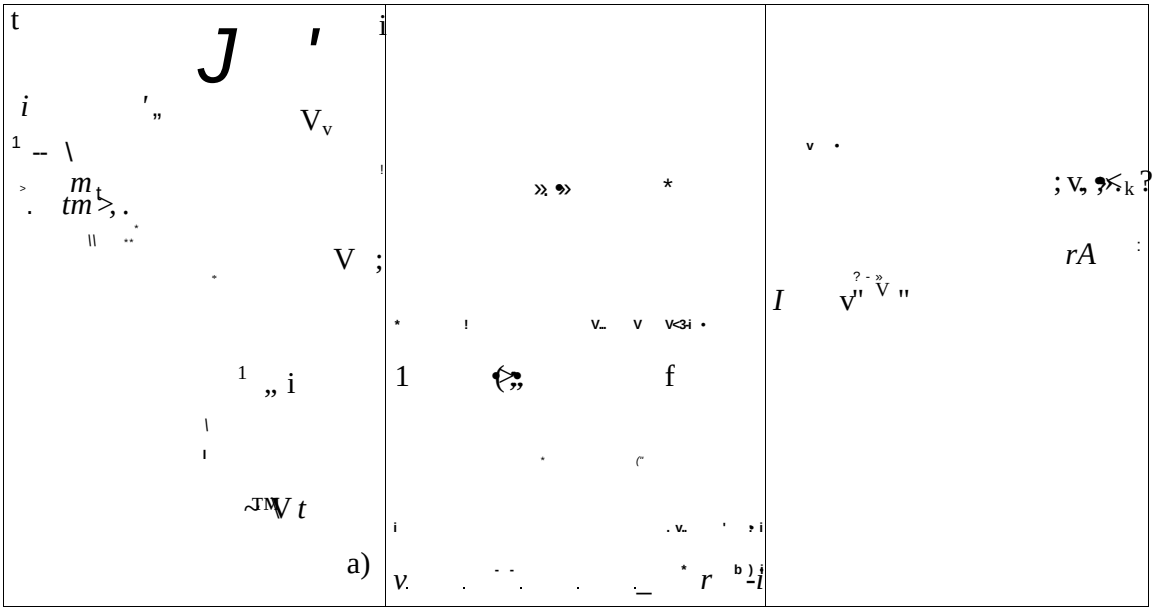

Figure 1. Distribution of a) *V. membranaceum,* b) *V. myrtilloides,* and c) *V. caespitosum.* (Vander Kloet 1988)

Vaccinium myrtilloides is found across North America at latitudes between 39°N and 61°N (Figure 1). It has been reported north to the Northwest Territories, south to West Virginia, west to Vancouver Island, and east to Labrador (Vander Kloet 1988, Vander Kloet and Hall 1981).

Vaccinium caespitosum is found across North America at latitudes between 20°N and 61°N (Figure 1). It has been reported north to Alaska, south to Mexico, west to the Queen Charlotte Islands, and east to Newfoundland (Vander Kloet 1988).

1.1.2 Habitat

Vaccinium species can be found growing in many different habitat types. *Vaccinium membranaceum, V. myrtilloides,* and V. *caespitosum* can all be found as forest understory shrubs and in non-shaded areas. In plots set up in western Alberta to study the distribution of

grizzly bear foods, *V. myrtilloides* was found more frequently in clear-cut areas compared to upland forests; however, *V. membranaceum* and V. *caespitosum* were found more frequently in the forests compared to the clear-cut areas (Nielsen *et al.* 2004). Kerns *et al.* (2004) looked at the frequency and density of three huckleberry species: *V. ovatum* Pursh(evergreen huckleberry), *V. parvifolium* Sm. (red huckleberry), and *V. membranaceum* in three different forest types, thinned, unthinned, and old growth in the Coast and Cascade mountain ranges of Oregon. In general, they found that *V. ovatum* was more prevalent in old growth and unthinned stands, whereas *V. parvifolium* was more prevalent in thinned stands. There was only one site that contained enough *V. membranaceum* to be used for analysis; this analysis showed that V. *membranaceum* plants were also more prevalent in the old growth and unthinned stands. The authors suggest that light levels, gap conditions, and available growing substrates may be among the factors influencing prevalence, although none of these variables were measured during the study. A survey of *V. myrtillus* L. (European bilberry), *V. vitis-idaea* L. (lingonberry), *V. uliginosum* L. (bog bilberry), and *V. oxycoccos* L. (small cranberry) in Sweden found that plants could be found growing along all parts of a gradient between forests and open bog (Eriksson and Froberg 1996). The plants found in the open bog conditions tended to be seedlings and younger individuals while the older individuals were found more often in the forest habitats. The seedlings and young plants were found to thrive on moist, high organic matter soils, which were more common in the open bogs. The authors could not explain why the older plants were found in different conditions.

Vaccinium species are also known to survive in harsh edaphic conditions. Erb *et al.* (1994) looked at genetic crosses of several *Vaccinium* species, including V. *corymbosum* and V. *myrtilloides*, and their ability to survive on high pH, drought stressed mineral soils in

Maryland. They found that the seedlings with the largest root systems at the time of transplanting showed the highest growth, measured as dry weight, at the end of the growing season. A cross between *V. myrtilloides* and *V. atrococcum* (A. Gray) A. Heller (black high-bush blueberry) was shown to be the most vigorous in the poor soil conditions. The authors speculate that *V. atrococcum* is providing most of the superior genes, but do mention that *V. myrtilloides* is one of the better adapted blueberries for mineral soils. In northern B.C., *Vaccinium myrtilloides* has also been shown to be more prevalent on low productivity soils than on higher productivity soils (Chen *et al.* 2004). In a test of four soil types from Maryland, V. *corymbosum* and various crosses of *V. corymbosum, V. darrowi* Camp. (Darrow evergreen blueberry), *V. angustifolium* Aiton (low-bush blueberry), V. *atrococcum,* and *V. ashei* J.M. Reade. (rabbiteye blueberry) were found to have deeper rooting and more even root distribution in the soils with the highest and lowest clay contents (Korcak 1986). After two growing seasons, the plants with the highest seedling volumes had been grown in the soil that had the lowest pH (3.6) and water-holding capacity (16%) and the highest electrical conductivity (21.4 meq/100 g) and percentage of sand (96%).

Overall, *Vaccinium* plants are able to grow in many different habitats. *Vaccinium membranaceum* and *V. caespitosum* plants prefer growing in undisturbed areas, such as mature forests. *Vaccinium myrtilloides* plants are better adapted to growing in disturbed areas, such as clear-cuts, thinned forest, or mineral soils.

1.1.3 Reproduction

Vaccinium plants can reproduce both sexually, through the production of seeds, and asexually, through rhizome production and layering. Calmes and Zasada (1982) looked at the reproductive traits of *V. uliginosum* growing in Alaska. They found that the plants spread

through layering, the growing of above-ground stems that then bend and are covered by organic matter. They found no evidence of seedlings growing in the wild and suggest that this is because there may not be any appropriate microsites for the seedlings to germinate and thrive.

Vander Kloet and Hill (1994) looked at six different *Vaccinium* species in Newfoundland and their presence in seed soil banks, and performed some germination trials using soil cores taken from field sites. They found that, although the plants produced many berries, their seeds were not well represented in the seed bank. In fact, only two species produced germinants from the soil cores at all. The authors also experimentally buried some seed for six years. Seeds exhumed every year continued to germinate, suggesting that the passage of time alone was not enough to reduce germination potential to zero. They have suggested that the lack of *Vaccinium* seeds in the soil cores may be due to fruit being eaten by birds or destroyed by fungal rot, but it is also possible that seed deposits were rare and the cores sampled had no *Vaccinium* seeds to begin with. Hill and Vander Kloet (2005) found that experimentally buried *Vaccinium* seed from Nova Scotia had an average longevity of 8.65 years, but some survived the entire 17 years of the study. Regression values suggested that the seed had the potential to survive over 20 years. The authors indicated that much of the variability could be explained through differences in climatic factors. They also noted that shorter-lived seeds were found more frequently within the seed bank than longer-lived seeds. A review by Whittle *et al.* (1997) also states that *Vaccinium* species, especially *V. myrtilloides* and V. *angustifolium*, have unknown longevity in seed banks and are known to reproduce mainly through asexual means.

Vander Kloet and Hill (2000) also studied the regeneration strategies of seven different *Vaccinium* species sharing the same geographical area in Newfoundland. They found that the three species most commonly found in the same locations (*V. angustifolium, V. boreale IN.* Hall & Aalders (northern blueberry), and *V. uliginosum*) had the most divergence in their dispersal mechanisms and germination characteristics, such as the timing of germination. Species with similar dispersal mechanisms or germination characteristics were generally found further apart from one another. As part of this study, the authors looked at the germination percentages of seeds in scats and decaying berries as well as cleaned seed. They found lower germination levels, ranging from 0-59% depending upon species, for the seeds from the scat and berries compared to those found for the cleaned seeds, which ranged from 5-88% depending upon species. The authors suggest that the cleaned seed germination levels, which are the levels commonly reported, overestimate the true germination that occurs under natural conditions. Overall, it seems clear that *Vaccinium* plants produce viable seeds and are able to regenerate from seeds given the proper growing conditions; however exactly what these growing conditions are is not well known. *Vaccinium* plants are also able to regenerate through rhizome production and layering, which is more commonly seen in natural populations.

1.1.4 Response to water stress

A variety of environmental stresses, such as drought, can limit *Vaccinium* growth. Glass *et al.* (2003) looked at drought tolerance in V. *angustifolium* under controlled conditions in Nova Scotia. The plants growing in water-excluded plots had access to measurably less soil moisture at the end of the experiment compared to the control plants. The drought-stressed plants had lower and more variable stem water potential as well as

lower photosynthesis and transpiration rates. Mortality rates were not reported for either the drought-stressed or control plants. In a subsequent study, the influence of drought stress on *V. angustifolium* in field plots in Nova Scotia was assessed (Glass *et al.* 2005a,*b)*. They found that fruit number was higher in irrigated plots than non-irrigated plots, although there was no effect on fruit weight, and surmise that, under drought conditions, the plants preferentially allocate photosynthates towards vegetative growth rather than reproductive growth. There were no differences between the irrigated and non-irrigated plants for gas exchange and stem water potential. The results indicated that all of the plants, including the irrigated ones, were under water stress as it was reported to be a very dry year. The plants in this study had their fruits harvested on a two-year cycle. *Vaccinium* plants in non-harvest years showed no differences in below-ground dry weight, however there were some differences in below-ground dry weight for plants in harvest years. The harvest year plants with one season of drought stress had the lowest dry weights, whereas the harvest year plants with two seasons of drought stress had the highest dry weights (2005b). This may indicate that, after one year of drought, the plants allocate more photosynthates into building below-ground structures, particularly roots, in order to access more water. Based on all of their studies the authors conclude that *V. angustifolium* can be considered a drought-tolerant plant. In summary, it would seem the *Vaccinium* plants, particularly *V. angustifolium,* are plastic in their response to water stress.

1.1.5 Response to disturbance

Ecosystem disturbances are common occurrences in areas where *Vaccinium* grow. Alaback and Tappeiner (1991) looked at V. *ovalifolium* Sm. (oval-leaf blueberry) seedlings in Alaska both before and following windthrow events. They found that seedling density

increased after the windthrow events and there was an exponential increase in shoot growth seen after a lag of 3-4 years, which appeared to be related to an increase in light levels. The *V. ovalifolium* plants were also found to allocate more carbon to root biomass after the windthrow events, suggesting that their strategy is to have energy stored in their rhizomes, allowing rapid re-sprouting when future disturbances occur.

Fire is another example of a common disturbance in areas where *Vaccinium* may grow. Hamilton (2006) looked at vegetation development over several years post-fire on mineral soil plots and forest floor plots north of McBride, B.C. She found that *V. membranaceum* mean percent cover increased after the burn on both soil types, but was generally higher on the mineral soil plots. The same results held true for a general *Vaccinium* sp. category, which included any species seen other than *V. membranaceum* or V. *ovalifolium. Vaccinium ovalifolium* also increased after fire, but was greater on the forest floor plots, possibly due to a pre-existing stand in those plots. The mean percent presence ratings of these three species/categories followed the same patterns as the mean percent cover, with the difference that for *V. membranaceum* and *V. ovalifolium,* the ratings seemed to peak 3-4 years after the burn and then began to decrease. The mean height of the vegetation also increased with years since fire. This time, both the *V. membranaceum* and *V. ovalifolium* grew taller in the forest floor plots, but the general *Vaccinium* sp. category still had taller plants in the mineral soil plots (Hamilton 2006).

A more extreme example of natural disturbances can be found in volcanic eruptions. Yang *et al.* (2008) looked at the genetic relatedness of *V. membranaceum* seedlings at Mount St. Helens, Washington, in the primary succession blast zone. They found 68 different genetic founders for the population, with many coming from the small pockets of plants

sprouting from roots and rhizomes that had survived the blast. The first seedlings are reported as being seen in 1990, 10 years after the eruption.

Forest management practices can also influence growth and prevalence of *Vaccinium* plants. Moola and Mallik (1998) looked at *V. myrtilloides* in plots in northern Ontario with full canopy coverage, partial harvest, and clear-cut harvest. They found that there were few differences between the partial harvest and clear-cut areas, except for stem age, with both partial harvest and full canopy areas having a mean stem age of 6.0 years and clear-cut harvest areas having a mean stem age of 3.0 years. The full canopy, or control treatment, was associated with lower light levels than either harvest type. This may have influenced the dry weight of stems, which was significantly lower in full canopy areas compared to either partial or clear-cut harvest areas. In a similar study, Atlegrim and Sjoberg (1996) looked at *V. myrtillus* in clear-cut areas, selective harvesting areas, and control unharvested plots in Sweden. They found that more sunlight reached the plants in the clear-cut and control areas; however ground cover of *Vaccinium* was highest in the control plots, possibly because there was no disturbance from harvesting equipment. Clear-cutting led to lower shoot survival and lower annual production of shoots as well. Assuming that the clear-cut treatments are similar, this suggests that *V. myrtillus* is a less plastic species than *V. myrtilloides,* or at least does not respond to clear-cut disturbance in the same way.

Haeussler *et al.* (1999, 2002) looked at the influences of mechanical site preparation on the plant communities of two sites in central and northern B.C. One of these sites was located near Bednesti Lake and included *V. myrtilloides* plants in the understory. The authors found that *V. myrtilloides* plants were highly sensitive to any disturbance by mechanical site preparation. In the 10 years directly following the disturbance, none of the

plants had re-established on the sites. They also found that removing the organic layers of a soil caused the plant community to shift to more seed-based species, such as lodgepole pine, rather than those that use vegetative propagation, such as *V. myrtilloides.* Overall, it would appear that disturbance that disrupts the soil structure, such as volcanic eruptions or mechanical harvesting, does not favour the growth of *Vaccinium* species. Conversely, disturbance that leaves the soil structure mainly intact, such as windthrow or low-intensity fire, does not hinder the growth of *Vaccinium* plants and in some cases may promote growth.

1.2 Vaccinium propagation

1.2.1 Propagation by seed

Vaccinium seeds are borne in fleshy berries on the plants in late summer. Each berry contains numerous seeds, which are about the size of the head of a pin. When propagating *Vaccinium* plants from seed, many factors must be taken into account. The age of the seed and storage methods are two of these factors. Darrow and Scott (1954) looked at the germination of *V. corymbosum* and *V. ashei* that had been stored under standard refrigeration conditions, approximately 4°C, for one to thirteen years. Overall, the *V. ashei* seeds germinated faster than the *V. corymbosum* seeds and generally had higher germination percentages as well. *Vaccinium ashei* seeds up to 12 years old still had germination levels in the 80% range. The *V. corymbosum* germination percentages were highly variable, with low percentages in the three year old seed and higher percentages for the five and nine year old seed. No explanation was given for this phenomenon. Barney (1996) also found that storage time did not influence the percentage germination in tests done on *V. membranaceum* from Idaho. In this case, seeds were stored at 0-4°C for one or six years, and then germination was compared to freshly sown seeds. Shafii and Barney (2001) conducted a study on the

influences of drying and cold storage on germination of *V. membranaceum* seeds collected in Idaho in 1989, 1995, and 1996. The treatments included fresh seeds, seeds air-dried for 7 days, seeds stored at 2-3°C for 1 year, and stored at 2-3°C for 7 years. The air-dried seeds had lower germination percentages than the fresh seeds, however those seeds that were then put in cold storage for one or seven years had germination percentages comparable to fresh seeds. This suggests that whatever dormancy may have been induced by drying the seeds was lost during the cold storage time. The two stored seed treatments also showed more variability in overall germination percentages than did the fresh or air-dried treatments (Shafii and Barney 2001).

Some types of seeds require different forms of pre-treatment, such as stratification (the exposure of seeds to either cold or warm conditions prior to germination), scarification (the wounding of the outer seed coat), or cleansing, in order to reach maximum germination potentials. Both *V. membranaceum* and *V. caespitosum* have been reported to require no scarification or stratification, but did require light exposure in order to germinate (McLean 1967). The influence of light exposure on seed germination was also examined by Barney (2003), who tested the influences of light, surface sterilization, and fungicides on the germination of *V. membranaceum.* The seeds were collected in Idaho in the summers of 1995 and 1996 and germinated without soil, on Petri plates. These tests showed that germination percentages were highest in seeds given 12 hr photoperiods and lowest in those kept in total darkness. The surface sterilization tests showed that seeds rinsed in 0.5% sodium hypochlorite (household bleach) did not have lower germination percentages than control seeds; however they did have shorter lag times between sowing and germination.

Seeds treated with the fungicides captan and mancozeb had reduced germination percentages compared to control seeds.

Further tests using *V. membranaceum* seeds from Idaho to determine the influence of cold stratification on germination found that the germination percentages were not statistically different between stratified and non-stratified seeds (Barney *et al.* 2001*a).* In contrast, a study coming out of Washington State found that *V. membranaceum* and *V. ovalifolium* seeds did need cold stratification to obtain maximum germination (Albright 2004), which is in direct opposition to the previous study. There is clearly a need for further studies in this area to clear up these opposing results. The influence of light, cold stratification, temperature, and over-wintering in berries on germination percentages was examined for *V. myrtillus, V. uliginosum,* and *V. vitis-idaea* seeds from Sweden (Baskin *et al.* 2000). The berries were collected from several different accessions for each species. They found that germination percentages were similar when seeds were kept in 20:10°C (day:night temperature) and 25:15°C for *V. myrtillus* and *V. uliginosum. Vaccinium vitis-idaea* had higher germination percentages at the highest temperature (25:15). All three species had lower germination percentages in the coldest temperature, 15:5°C. Seeds of *V. myrtillus* and *V. vitis-idaea* did not germinate in the dark; however *V. uliginosum* seeds germinated at higher percentages in the dark than in the light. The authors found that cold stratification increased germination percentages at the coldest temperatures, but decreased germination percentages at the higher two temperatures for *V. myrtillus,* and increased germination percentages at all temperatures for the other two species. Seeds extracted from over-wintered fruits of *V. myrtillus* and *V. vitis-idaea* reached maximum germination at about six weeks from sowing (Baskin *et al.* 2000). Overall, exposure to light seems to be beneficial to the

seed germination of most *Vaccinium* species. Studies examining the effects of cold stratification on seed germination produce varying results and more work should be done in this area.

The medium used to grow seedlings can also influence their germination and growth. Traveset *et al.* (2001) looked at the influence of manure composition on the seedling emergence and growth of *V. ovalifolium* in Alaska. *Vaccinium* seeds often travel through the gastro-intestinal tract of birds and mammals when the berries are consumed. The seeds were growing in pots of peat mixed with grizzly bear feces either mainly composed of animal matter or plant matter, or a control group which was just peat. The presence and composition of the manure had no influences on seedling emergence of *V. ovalifolium*, which was 30-40%; however growth was shown to be higher in both of the manure treatments, probably due to increased nutrients. These results suggest that there is no benefit to the germination of *Vaccinium* seeds from growing in manure. The seeds used had not actually traveled through the gastro-intestinal tract, so no conclusions can be reached about the influences of such a passage on the germination of *Vaccinium* seeds.

1.2.2 Shoot cuttings

In cases where consistent features are desirable in the new plants vegetative propagation, usually using shoot or rhizome cuttings, is preferred over sexual propagation using seeds. Kender (1965) looked at the effect of seasonality, location on the plant, and rooting hormones on the success of softwood cuttings of *V. angustifolium* in Maine. He found that seasonality was an important factor in rooting success, with cuttings taken in late June and early July having better success than those taken later in the growing season. Cuttings taken directly from shoots originating from the rhizomes rooted better than those

taken from shoots originating from aerial stems. It was also found that the clone of the plant made a difference in the rooting success, suggesting a genetic component to the success of the cuttings. The rooting hormone that was used had no influence on the rooting success of the cuttings. Current protocol for *V. membranaceum* shoot cuttings calls for 2000 ppm indole-3-butyric acid (IBA) to be applied to the shoot prior to planting; however, success rates for this method are only reported to be *8%* (Evans *et al.* 2001). Both temperature and photoperiod had a significant effect on the development of rhizomes on softwood cuttings of *V. angustifolium* taken in Maine (Kender 1967*a).* Higher temperatures resulted in more rhizome development than lower temperatures. Longer photoperiods also resulted in more rhizome development for the cuttings. The higher temperatures also seemed to produce a more marked difference in the differences between the photoperiods, as seen by a significant interaction term in the analysis. When planted into field conditions in row culture, rooted softwood cuttings of *V. angustifolium* took longer to spread than either seedlings or transplanted sod patches from established stands; however, all three propagation methods formed commercially acceptable fruit-bearing plants within three years (Kender 1967b).

1.2.3 Rhizome cuttings

Rhizome cuttings are also a form of vegetative propagation that can result in plants identical to the parent plant. Kender (1969) looked at the influences of plant growth regulators and temperature on the propagation of *V. angustifolium* from Maine by rhizome cuttings. He found that the application of gibberellic acid (GA) or IBA to the rhizomes actually reduced the sprouting, whereas these compounds are considered to increase rooting when used with stem cuttings. The author also found that higher temperatures on the growing bench reduced sprouting for the rhizome cuttings. He suggests that the higher

temperatures may put the rhizome into a "rest" period, which naturally occurs in June and July, and make it more difficult for them to sprout. The study also looked at the effect of day length, and it was concluded that there was no effect of day length on rhizome sprouting. This, again, is the opposite result of what he earlier found for *V. angustifolium* softwood cuttings. Early spring or early fall is reported to be the best time to collect rhizomes for propagation. Rhizome cuttings planted directly into field conditions have been found to have very high mortality rates, and therefore were not considered to be a very practical method of propagating (Kender 1967b).

1.2.4 Micropropagation

Another technique for asexually propagating plants is micropropagation, also known as tissue culture or leaf culture. For *Vaccinium* species, several micropropagation studies have been published. Barney (1999a) grew *V. deliciosum* Piper (cascade huckleberry), *V. membranaceum,* and *V. ovalifolium,* collected from western Washington and Oregon, using tissue culture methods with different levels of IBA applied to the microshoots. He found that 3 mg/L of IBA was the most effective level for survival, root production, and shoot production. He suggests that these results are especially important with regard to *V. membranaceum,* which roots poorly from stem cuttings but is desired for domestic production. A more recent study (Barney *et al.* 2007) looked at the influences of two different tissue culture rooting media at two different concentrations on the growth of *V. membranaceum, V. deliciosum,* and V. *ovalifolium.* They found that *V. membranaceum* grew better on woody plant medium at both full and half strength compared to the classic Murashige and Skoog medium (Barney *et al.* 2007).

Plants grown using micropropagation methods are often compared to those grown using other asexual propagation methods. *Vaccinium vitis-idaea* plants grown by leaf culture in Newfoundland had more stems, branches, and rhizomes than shoot cuttings of the same species (Debnath 2006). Adding IBA to the leaf culture influenced the shoot morphology and rhizome morphology, but not the number of rhizomes being formed. The study also revealed that specific cultivars, and therefore genotypes, had significant effects on the outcome of the experiment. Morrison *et al.* (2000) looked at the comparative growth of *V. angustifolium* micropropagated plantlets, rooted softwood cuttings, and seedlings with a goal of using them to fill in gaps in existing low-bush blueberry field sites. The micropropagated plantlets outperformed the rooted cuttings when looking at the number and size of rhizomes produced per plant after two growing seasons (Morrison *et al.* 2000). In another experiment, over one growing season, they found that the seedlings actually had the highest number of rhizomes produced per plant compared to plantlets and cuttings. This must, of course, be weighed against the slow growing time of the seedlings when compared to the use of micropropagation for producing plants of the same size. The authors were using rhizome number and size as a measure of the ability of the plants to spread and fill the gaps in existing fields. Overall, micropropagated plants are a viable alternative to using seeds or cuttings for propagating *Vaccinium* plants if the technology is available.

1.3 Ericoid mycorrhizas

1.3.1 Mycorrhizal structure

Ericoid mycorrhizas are symbiotic relationships between plants in the order Ericales and certain groups of soil fungi. The structures associated with ericoid mycorrhizas are constant across all of these relationships and can be found within the hair roots of the host

plants. Fungal hyphae penetrate the walls of the epidermal cells and form dense coils within these cells. Mycorrhizal colonization causes the walls of the epidermal cells to thicken, which provides protection for the fungal hyphae (Massicotte *et al.* 2005, Peterson *et al.* 2004, Smith and Read 2008).

1.3.2 Fungal partners

The fungal partners of ericoid mycorrhizas have traditionally not been very well identified. Xiao and Berch (1992) did a study looking at the mycorrhizal fungal partners of *Gaultheria shallon* Pursh plants collected on Vancouver Island (B.C). They took roots from wild stands and isolated fungi from them, and then re-inoculated young seedlings grown from purchased seeds. Several morphologically different cultures were documented, including *Oidiodendron maius* Barron and many that they were unable to identify. Rice and Currah (2005), in their review paper, also identified *O. maius* as an ericoid mycorrhizal fungus. In a laboratory experiment, Xiao and Berch (1995) inoculated roots of *G. shallon* with fungal strains known to form ericoid mycorrhizas with other ericaceous species. Of the 14 fungal strains tested, five formed typical ericoid mycorrhizas with *G. shallon*, suggesting that the fungal partners in ericoid mycorrhizas are not host-specific. Xiao and Berch (1996) also sampled roots of *G. shallon* from two forest types on Vancouver Island. They found over 85% of the roots to be colonized. They cultured the fungi from the roots and were able to group them into four species based on colony morphology and asexual fruiting body characteristics. These species included *O. maius, Acremonium strictum* W. Gams., and two unidentified species that did not produce spores. The levels of colonization did not differ between the two forest types examined.

In total, mycorrhizal fungi found on roots of *G. shallon* from Vancouver Island showed a great diversity of fungi, including 5 species and 2 polyphyletic assemblages including many unidentifiable species (Berch *et al.* 2002). More recently, Vohnfk *et al.* (2005) inoculated *Rhododendron* sp. plantlets with *O. maius* and *Rhizoscyphus ericae* (D.J. Read) W.Y. Zhuang & Korf, one of the most common ericoid mycorrhizal fungi, and found that *O. maius* did not colonize as well as *R. ericae,* with fewer and looser hyphal coils found in the epidermal cells. This suggests that there may actually be some differences between the ability of certain ericoid mycorrhizal fungi to colonize different plant species.

1.3.3 Distribution

Ericoid mycorrhizas can be found in a number of different environments. Treu *et al.* (1996) did a survey of plants in Denali National Park and Preserve in Alaska to document the extent of mycorrhizal associations. Eight different ericaceous species were collected. All of these species except *Arctostaphylos alpina* (L.) Spreng. showed typical ericoid mycorrhizal relationships. The *A. alpina* showed arbutoid mycorrhizas, which are typical for that genus. Haselwandter (1979) collected ericaceous plants from different altitudes in the Austrian Alps. Using glucosamine levels as a proxy of mycorrhizal infection, he measured the colonization levels of the roots systems from different altitudes. By this measure, mycorrhizal infection levels were lower in higher altitudes than in lower altitudes. The author suggests that this is because of the shorter growing season leading to less C production for the fungal partner. Overall plant biomass was positively correlated with glucosamine levels for all ericaceous plants at all altitudes, suggesting that the presence of ericoid mycorrhizas aids in plant growth.

1.3.4 Soil Conditions

Ericoid mycorrhizas often exist in harsh environmental conditions. In their review paper, Cairney and Meharg (2003) discuss how the fungal partners confer their plant hosts with resistance to environmental stresses by mobilizing soil nutrients that would otherwise be unavailable. The fungal partners may also confer a degree of heavy metal resistance to their host plants by preventing the metal ions from reaching the plant tissues. Bradley *et al.* (1981, 1982) tested the survival and yield of two populations of *Calluna vulgaris* L. in the presence of copper and zinc. The two populations were collected from seed from a site contaminated with heavy metals in Wales and a non-contaminated site in England. The seeds were then inoculated with *R. ericae* to determine any influences of mycorrhizal colonization. The mycorrhizal seedlings had significantly higher yield than the non-mycorrhizal seedlings. There was also considerably lower heavy metal content found in the shoots of the mycorrhizal seedlings. Read (1984) conducted a study with soils from *Calluna* heathlands in England as well as grass seedlings. The native ericaceous plants were better able to survive in the heathland soils compared to the grass seedlings. There was also evidence that the ericaceous plants may have been contributing to the maintenance of the harsh low pH and high organic acid conditions. This ecological process might function in excluding other non-ericaceous plants from the area. Leake *et al.* (1989) also performed a series of laboratory tests concerning the detoxifying abilities of ericoid mycorrhizas. They found that some of the fungal partners were able to help the plant survive in conditions that it would be unable to inhabit otherwise.

Rhizoscyphus ericae isolated from C. *vulgaris* roots has been found to be capable of reducing arsenate to arsenite, therefore functioning as a type of filter for the plants it was

associated with (Sharpies *et al.* 2000a,*b).* This reducing capability was more prevalent in *R. ericae* isolated from plants growing in contaminated soils than non-contaminated soils, suggesting that heavy metal resistance may be a locally adaptive trait. Martino *et al.* (2000) isolated two strains of *O. maius* growing on the roots of *V. myrtillus* in a contaminated soil in Poland. They tested the mycorrhizal abilities of the isolates, and they both proved to be mycorrhizal fungi. These isolates were then compared to isolates taken from non-contaminated soil and the ability of both sets of isolates to grow in the presence of zinc ions was tested. The isolates from the contaminated soil showed better growth and survival in the presence of Zn, which supports the idea that heavy metal resistance is a locally adapted trait (Martino *et al.* 2000).

Monni *et al.* (2000a) did an experiment in Finland to test the tolerance of *Empetrum nigrum* L., an ericaceous shrub, to copper and nickel. The results showed that *E. nigrum* was more tolerant to these heavy metals than hyperaccumulation should account for. The authors suggest that there must be an internal mechanism for the demonstrated tolerance, which could be similar to the arsenate reduction described above by Martino *et al.* (2000). Monni *et al.* (2000b) also tested the copper resistance of *C. vulgaris* seedlings collected from different distances from a copper-nickel smelter in Finland. Unsurprisingly, they found that higher levels of copper resulted in lower growth parameters for the seedlings. The distance from the smelter that the seeds were collected from did not seem to have any effect on the outcome of the trials, which would go against the idea of heavy metal resistance being a locally adapted trait.

Soil nutrients are one of the factors that can affect mycorrhizal colonization in *Vaccinium* plants. Turnau *et al.* (1992) looked at the influences of fertilization in an oak-pine

forest in southern Poland over three years. Plots were treated with nitrogen (N), phosphorus (P), and potassium (K) fertilizer, while control plots were left untreated. The plant species were surveyed and their mycorrhizal status was assessed. The ericoid plants, which included *V. myrtillus,* were negatively affected by the fertilization, decreasing in number. Several arbuscular mycorrhizal species increased in number with fertilization, while still other species showed no differences at all. This suggests that ericaceous plants may have lower competitive abilities in less harsh edaphic conditions. *Vaccinium corymbosum* plants from two existing plantations in Italy, each about twenty years old, showed significant differences in mycorrhizal colonization rates (Czesnik and Eynard 1989, Eynard and Czesnik 1989). Higher mycorrhizal colonization levels were found in the site that had been mulched over the past 20 years, which had higher levels of organic matter, N, P, as well as a lower pH. There was also a significant cultivar difference in both mycorrhizal colonization levels and vigour, suggesting than there may be a considerable genetic component to these measures, but also that past soil agricultural practices influence the outcome of mycorrhizal colonization.

Vaccinium corymbosum plants from commercial fields in Pennsylvania had the influences of amendment, mulching, and N levels on mycorrhizal colonization and plant vigour examined over a two year growing season (Goulart *et al.* 1995*a)*. At the end of the two years, unmulched plants showed decreasing mycorrhizal colonization with increased N applications and mulched plants did not show any consistent trends. Plants which had been mulched and/or amended generally showed more growth and vigour than those that were not. Plants without mulching and/or amendments had lower canopy volumes and lower mycorrhizal colonization levels with increasing nitrogen applications. In another study, Goulart *et al.* (1997) looked further at the influences of mulch amendments and N on the

characteristics of *V. corymbosum* plants grown in mineral soil plots. Mycorrhizal colonization did not affect the growth and yield of these crops, however mulching and amending were associated with increased growth and yield. When there were no amendments, N level was correlated with mycorrhizal infection. The mycorrhizal colonization levels of *V. corymbosum* and *V. angustifolium* root systems in both commercial and native populations in Pennsylvania were found to vary with cultivar, site, and time of sampling (Stevens *et al.* 1997). The time of sampling may be attributable to seasonal fluctuation in fungal availability. The native populations had higher levels of fungal colonization than the commercial levels, which could be attributable to the lower pH and nutrient levels usually found on these sites. The authors also re-cultured and identified mycorrhizal fungi from the collected roots. The variability in fungal taxa found was equal between the commercial and native sites. There was also no difference in the variability of fungal taxa found between the two different *Vaccinium* species examined.

1.3.5 Environmental Conditions

Environmental conditions can also play an important role in the mycorrhizal colonization of plants. Jeliazkova and Percival (2003) performed a water-exclusion experiment on mature plants of *V. angustifolium* in Nova Scotia to study the effects this had on mycorrhizal colonization. Analysis showed that the exclusion of rainfall negatively affected the total soil moisture, but had no influence on mycorrhizal colonization. The authors suggest that the plants may be reliant upon their rhizomes to get them through times of drought stress.

Olsrud *et al.* (2004) looked at several different ericaceous shrubs, including some *Vaccinium,* and their responses to changing environmental conditions in Sweden. The

authors used ergosterol content as a proxy measure of mycorrhizal colonization and found that the levels in the roots changed in response to changing CO2 levels. The ergosterol content of the roots increased with increasing photosynthetic levels of the plants. The correlation between ergosterol content and ericoid mycorrhizal colonization or dark septate endophyte presence was examined in several ericaceous shrubs in Sweden; however, there was no relationship found between any of the variables (Olsrud *et al.* 2007), so this puts into question the results in the previous study.

1.4 Use of mycorrhizal inoculation in Vaccinium propagation

1.4.1 Methods of inoculation

There are several different methods of inoculating plants with mycorrhizal material. One of the simplest methods is to use soil from established *Vaccinium* stands, assuming that there will be fungal propagules contained within the mixture (Powell and Bates 1981, Powell and Bagyaraj 1984). Another method is to use laboratory grown fungal cultures, usually processed into a solution, and add these fungi directly to the plants. Goulart *et al.* (1995*b)* looked at three different methods of inoculating *V. corymbosum* using Petri dishes with three different kinds of agar, growth pouches with mycelial dips, solids, or agar plugs, and inoculation in pots, which was done as a mycelial dip before transplanting into a sand-based medium. They found no significant differences in the levels of mycorrhizal colonization between the treatments 20 days after initial inoculation. The colonization levels ranged from 0.36-4.3% of epidermal cells being colonized (Goulart *et al.* 1995*b).*

Fungal inoculants can also be purchased as commercial solutions or mixes. These commercial inoculants vary in quality and effectiveness. Haynes and Swift (1985) compared the mycorrhizal infection levels of *V. corymbosum* plants from tissue culture, approximately

7 cm tall, that had been inoculated with a laboratory grown *R. ericae* culture and a commercial inoculant (Mycoaid®) containing *R. ericae* propagules. They found that 16 weeks after transplanting, the plants that had been inoculated with the *R. ericae* culture had much higher mycorrhizal infection levels that those inoculated with the commercial inoculant.

1.4.2 Effects of inoculation on mycorrhizal colonization

Applying any form of mycorrhizal inoculant to a plant does not guarantee that the mycorrhizal colonization will occur; just as not inoculating a plant does not guarantee that the plant will not have any mycorrhizal colonization. In some studies, inoculation is clearly positively correlated with mycorrhizal infection levels. Hardwood cuttings of *V. corymbosum* from New Zealand that had been inoculated with either mycorrhizal peat from underneath established plants, *R. ericae* pure culture at 5 mg/cutting, *R. ericae* pure culture at 50 mg/cutting, or left uninoculated showed clear differences in mycorrhizal colonization levels (Powell and Bagyaraj 1984). The mycorrhizal peat produced the highest level of mycorrhizal colonization (36-40%) and root formation. Uninoculated cuttings had colonization levels of 4-12% and cuttings inoculated with *R. ericae* pure culture at 5 mg/cutting and 50 mg/cutting had colonization levels of 5-18% and 6-15%, respectively. The addition of fertilizer to the plants did not affect the colonization levels. In another component of the experiment, softwood cuttings were uninoculated, fertilized, inoculated only, or inoculated and fertilized. Again, it was found that the application of fertilizer did not detrimentally affect the mycorrhizal colonization levels, which were higher in the inoculated plants (17-23%) than in the uninoculated plants (2-7%). In the test of *V. corymbosum* using commercial inoculants, laboratory grown cultures and control plants, the control plants were

always found to have no colonization, whereas the inoculated plants did show mycorrhizal colonization with levels of 48-69% (Haynes and Swift 1985). *Vaccinium corymbosum* seedlings inoculated with *R. ericae* and grown in an unsterilized peat/sand mixture were also found to be colonized, with levels of 19-52%, when examined 67 days after the initial inoculation (Powell 1982).

Yang *et al.* (1998) tested how effective soil fumigation with a fungicide was at keeping *V. corymbosum* plants non-mycorrhizal. Outdoor plots were set up in Pennsylvania, half of the plots were amended with aged sawdust and half were left without amendment. The plots were then fumigated and planted, with half the plants being inoculated with an O. *maius* suspension and half inoculated with sterile water. Mycorrhizal infection was assessed each fall for two growing seasons. In the first growing season, the non-inoculated plants only had minimal mycorrhizal infection compared to the inoculated plants. After the second growing season, the non-inoculated plants had infection levels comparable to the inoculated plants after the first season. The inoculated plants still showed higher levels of colonization than the non-inoculated plants after two growing seasons. This suggests that naturally occurring inoculum had re-established in the plots after one growing season. The presence of naturally occurring inoculum was also demonstrated in a trial of *V. corymbosum* field plants that were inoculated and measured for fruit yield after 2 years. The inoculation was positively correlated with increased fruit yield; however, the non-inoculated plants were colonized to the same level as the inoculated ones (Powell and Bates 1981). *Vaccinium angustifolium* tissue culture plantlets from Maine were inoculated with one of three mycorrhizal fungal isolates or left uninoculated. The plantlets showed colonization rates

ranging from rare to highly frequent, but the colonization rates did not appear to be related to the inoculation process at all (Smagula and Litten 1989).

1.4.3 Effects of inoculation on plant growth and development

As mycorrhizal relationships are common in native *Vaccinium* plants, it is reasonable to assume that the inoculation of plants would be beneficial. Smagula and Litten (1989) looked at the influence of mycorrhizal inoculation on the growth of tissue culture plantlets of *V. angustifolium* in Nova Scotia. They found that inoculated plants showed no growth differences compared to uninoculated plants in almost all cases. The application of fertilizer did, however, affect growth parameters. The different cultivars of *V. angustifolium* also influenced the mycorrhizal colonization levels. The effects of mycorrhizal inoculant added prior to rooting of micropropagated *V. angustifolium* and the effect of the irradiation of peat mixes on growth and mycorrhizal colonization was examined by Litten *et al.* (1992) in Nova Scotia. After 167 days, all of the cuttings in inoculated unirradiated peat grew better than those in uninoculated unirradiated peat. The results for the irradiated peat did not show any trends.

Another study looking at the influence of mycorrhizal fungi as well as soil pH on the growth of *V. corymbosum* was done by Reich *et al.* (1982) in Maryland. The higher pH level was associated with lower growth of the blueberry seedlings; however, the pH levels did not seem to be associated with mycorrhizal colonization levels at all. After nine months in greenhouse conditions, plant root colonization was measured using microscopy. The authors speculate that the high pH may be inhospitable to native mycorrhizal fungi.

Vaccinium corymbosum plants grown in greenhouse conditions in Pennsylvania, using tissue culture propagation, were either inoculated with an *O. maius* suspension that had

been prepared in the laboratory or left uninoculated before being transplanted to outdoor plots (Yang *et al.* 2002). All plots were treated with a fungicide prior to inoculation to remove any naturally occurring inoculant. There were three soil amendment treatments that occurred prior to planting: forest litter, sawdust, and no amendment. The forest litter came from an oak forest with ericaceous plants in the understory. At the end of the experiment, the dry weight of the plants was measured. Mycorrhizal inoculation led to significantly higher total, shoot, and root dry weights. Pre-planting amendment with forest litter also led to significantly higher dry weights. Plants amended with sawdust had the lowest dry weight.

Scagel (2005) was working in Oregon with potted *V. corymbosum* plants. Three fungal species were used to inoculate the plants, as well as an uninoculated control group. Plants were also fertilized with either organic or inorganic fertilizer, and seven different cultivars were used. Plants that had been inoculated with any of the fungal species had higher growth rates and mycorrhizal infection rates than those in the control group. This held true for both of the fertilizers. Inoculated plants seemed to grow best with organic fertilizer, while the uninoculated plants seemed to grow best with inorganic fertilizer.

1.5 Study objectives

Past studies on *Vaccinium* plants have not included many species native to north-central British Columbia, such as *V. membranaceum, V. myrtilloides,* and *V. caespitosum.* Those studies that do include these species do not give a clear picture of how to maximize growth and survival of these plants under cultivation. The first objective of this study was to develop propagation protocols for *V. membranaceum, V. myrtilloides,* and *V. caespitosum* being grown in greenhouse conditions. This was accomplished by testing different germination conditions for *Vaccinium* seeds and comparing them to success levels obtained

with hardwood and rhizome cuttings of the same species. Once a method for growing sufficient numbers of plants was obtained, the next objective was to develop the optimal conditions for transplanting the young *Vaccinium* plants into outdoor conditions. This was done by testing the influences of mycorrhizal fungal inoculation and soil amendment on the growth and survival of *Vaccinium* seedlings over one field season. Table 1 gives an outline of all of the trials undertaken over the course of this study.

Table 1. *Vaccinium* propagation and transplanting trials

Date	**Trial**
Fall 2006	Seed storage time and germination trials
Spring 2007	Hardwood cutting trial
Fall 2007	Seed germination trial
Spring 2008	Rhizome cutting trial
Summer 2008	Field trial with fungal inoculation and soil amendment
Summer 2008	Supplemental field trial with only soil amendment
Fall 2008	Seed germination trial
Fall 2008	Growth analysis trial with fungal inoculation

2.0 Propagation of *Vaccinium membranaceum, V. myrtilloides,* and *V. caespitosum* by seed, hardwood cutting, and rhizome cutting methods

2.*1* *Introduction*

Plants in the order Ericales, including *Vaccinium* species, occur worldwide, often growing under harsh, nutrient-poor conditions. There is great demand for the inclusion of berry-producing plants such as *Vaccinium* spp. in forest restoration and post-industrial reclamation efforts, due to their value to wildlife and traditional users of the land. However, the biology and propagation requirements of northern *Vaccinium* species and their potential for use in reclamation, restoration, and horticulture remain largely unexplored.

Propagation by seeds is one of the oldest and most commonly used methods of shrub propagation. Propagating by seed allows for the maintenance of genetic variability, and hence the potential for future genetic adaptations in lines of plant material (Rose *et al.* 1998). Seeds can be collected from many sources including seed orchards, nursery stock, and native populations. Propagation by seeds is one of the most commonly used methods of propagating *Vaccinium* plants. *Vaccinium* berries are collected in late summer or early fall. The amount of berries produced by the plants can vary greatly year to year depending upon weather conditions during the growing season (Small and Catling 2005). Most species of *Vaccinium* require no pre-germination treatments, however *V. uliginosum* has been reported to benefit from a period of cold stratification as well as a gibberellic acid (GA) rinse (Barney 2006). Studies have suggested that *Vaccinium* seeds can be stored for up to 13 years without losing viability (Darrow and Scott 1954; Dirr and Heuser 1987).

Propagation from vegetative cuttings is one of the most economical techniques for shrub propagation (Dirr and Heuser 1987). The resulting plants are genetic clones of the

parent plant and will keep all its characteristics, but with none of the incompatibility issues noted when using grafting or budding techniques (Dirr and Heuser 1987). When propagating plants using cuttings, both stem cuttings and root cuttings can be used. Limited success has been reported using stem cuttings for *V. membranaceum* (Evans *et al.* 2001); however other *Vaccinium* species have been reported to root very successfully using stem cuttings (Kender 1967b). Several *Vaccinium* species have been reported to sprout from rhizome cuttings taken while the plant is dormant (Rose *et al.* 1998). The use of both stem and root cuttings in the propagation of *Vaccinium* plants is an area that needs further research.

To address some of the gaps in native *Vaccinium* propagation knowledge, the research reported here consisted of testing and optimizing the propagation and culture of *V. membranaceum*, *V. myrtilloides*, and (when available) *V. caespitosum*, using seeds, hardwood cuttings, and rhizome cuttings. Seeds were tested for the influences of storage time on germination percentages and germination speed. It was hypothesized that the amount of time the seeds had been stored would have an effect on their total germination percentages. Hardwood cuttings were tested for the influences of rooting substrate, bottom heat, and rooting hormone formulation on rooting percentage, above-ground vigour, and total number of roots per cutting. It was hypothesized that each of the above-mentioned factors would affect the rooting, above-ground vigour, and number of roots per cutting of the hardwood cuttings. Rhizome cuttings were tested for the influences of planting medium and bottom heat on total sprouting percentage. It was hypothesized that different media and bottom heat applications would have an effect on the total amount of sprouting.

2.2 Methods and Materials

2.2.1 Seed trials

The seeds of *V. membranaceum, V. myrtilloides,* and *V. caespitosum* used in the trials were extracted from multiple provenances of locally collected berries. Berries were collected during the 2005, 2006, 2007, and 2008 growing seasons. Exact berry collection locations and dates are given in Appendix 1. One provenance of each of the three species was collected in 2005 and 2006. Three provenances each of *V. membranaceum* and *V. myrtilloides* and two provenances of *V. caespitosum* were collected in 2007. Two provenances each of *V. membranaceum* and *V. myrtilloides* were collected in 2008.

For each trial, berries were stored at 4°C until the seeds were extracted from the berries using a protocol modified from Barney (1999b), Stevens and Darris (2000), Rose *et al.* (1998), Dirr and Heuser (1987), and Georgeson Botanical Garden (2007). The blades of an Oster® 12-speed kitchen blender were wrapped in a thick layer of electrical tape to prevent damage to the seeds. Berries and water were added to the blender in a 1:1 ratio and blended at medium speed for several 30-second bursts, until the berries appeared well macerated. The resulting slurry was strained to remove the berry pulp and allowed to sit for 1-2 minutes to allow the viable seeds to settle to the bottom. The water was then decanted off of the seeds and they were left to air dry.

In the fall of 2006, trials were set up to test the effects of seed storage time on germination percentages of *V. membranaceum, V. myrtilloides,* and *V. caespitosum.* Each of the three *Vaccinium* species had freshly extracted seeds, seeds that had been extracted and air dried for 48 hours, and seeds that had been stored for approximately one year tested for germination percentages, for a total of nine different treatment combinations. Seeds were

surface sown on a 50:50 (by volume) peat:sand growing medium. Nine rows of 20 seeds each were sown per tray, with each row consisting of a different storage treatment x *Vaccinium* species combination. Treatment combinations were randomized within each tray and five trays were sown in total. Trays were bottom-soaked for 10 minutes after seeding, and then allowed to drain. Growing trays were covered with clear plastic domes and kept under greenhouse conditions with a 21°/10°C day/night temperature and 15 hr daylight photoperiod. The trays were watered every 7-10 days as necessary. Germination numbers were recorded 64 days after seed sowing.

In the fall of 2007, 10 berries from each provenance collected were dissected and the number of seeds per berry was recorded. Seeds were allowed to air dry for 24 hrs, and stored at 4°C for 25 days prior to sowing. Five replicates of 100 seeds each per provenance (with the exception of *V. membranaceum* from Teapot Mountain and *V. caespitosum* from Mackenzie, which were not available) were surface sown on to a 50:50 peat:sand growing medium and kept under greenhouse conditions with a 21710°C day/night temperature and 15 hour photoperiod. Seed trays were covered with clear plastic domes, placed on capillary matting, and watered every 7-10 days as necessary. Germination numbers were monitored weekly until 93 days after sowing. Total germination percentage and number of days, to the nearest weekly observation, required to reach 50% of total germination were recorded.

In the fall of 2008, germination trials for *V. membranaceum* and *V. myrtilloides* seeds were conducted in the laboratory. Three replicates of 100 seeds each were placed on filter paper that had been moistened with deionized water inside plastic Petri dishes. The dishes were sealed with Parafilm and kept at room temperature (20°-23°C) in natural daylight conditions during the September-December time period. Seeds were examined for

germination on a weekly basis and germinated seeds were removed from the Petri dishes. New Parafilm was applied to dishes weekly and filter paper was re-moistened as necessary to prevent drying. Germination was monitored weekly and the final germination count was done 100 days after the seeds were initially plated. Total germination percentage and number of days required to reach 50% of total germination were recorded.

Statistical analysis

Germination experiments were set up as a randomized complete block design, with each tray serving as a complete block or set of replicates. Individual data observations consisted of final germination count observed for each row of 20 seeds for the 2006 trials and final germination count observed for each replicate of 100 seeds for the 2007 and 2008 trials. The effects of species and seed storage time on germination percentage were analyzed using two-way analysis of variance (ANOVA) for the 2006 trials. The effects of species and provenance on germination percentage and the number of days required to reach 50% of total germination were analyzed using two-way ANOVA for the 2007 and 2008 trials. All data throughout the chapter were examined at a significance level of 0.05. An angular transformation (arcsine(Vx)) was used on the germination percentage data to ensure equal variance and normal distribution of the residuals. Differences between individual treatment combinations were analyzed using Tukey's post-hoc test with Bonferroni adjustments. Number of seeds per berry counts were analyzed for differences between species and accessions using two-way ANOVA. A square-root transformation was used on the data to ensure equal variance and normal distribution of the residuals. Differences between individual treatment combinations were analyzed using Tukey's post-hoc test with Bonferroni adjustments. Post-hoc power analyses were performed on all trials to determine the

probability of type II errors. All statistical analyses throughout this chapter were performed with the R statistical computing program (R Development Core Team, 2007).

2.2.2 Hardwood cutting trials

Trials were set up to test the effects of substrate, bottom heat, and hormone formulation on the rooting of hardwood cuttings of *V. membranaceum* and *V. myrtilloides.* All cuttings were collected from local native stands within the city limits of Prince George, British Columbia, in early February 2007 and cut into sections 8-10 cm in length. The cuttings were treated with either 20, 000 ppm indole-3-butyric acid (IBA), 10, 000 ppm naphthalenacetic acid (NAA), or a combination of 20, 000 ppm IBA and 10, 000 NAA. Cuttings were then planted in growing trays containing either rockwool or a 50:50 (by volume) peat:perlite growing medium. Half of the growing trays, which each contained an equal number of the two species and three hormone treatments in a random placement, were then placed on heating mats set to 27°C while the other half were left without bottom heat. In total, there were 240 cuttings (2 species x 2 substrates x 3 hormone treatments x 2 heat treatments x 10 replicates). Growing trays were covered with clear plastic domes and kept under 21°/10°C day/night temperature with unsupplemented natural light conditions. These trials were carried out over the February-March time period. Trays were watered every 7-10 days as necessary and the plastic domes were removed for approximately six hours every three days to allow for air circulation.

On the 62nd day after planting, the cuttings were evaluated for rooting. The evaluation included categories for cuttings with no roots and cuttings with roots present. Cuttings with roots present then had the number of roots per cutting counted. Above-ground vigour of the cuttings, categorized as dead or alive, was also recorded.

Statistical analysis

Hardwood cutting experiments were set up as a randomized complete block design, with each tray serving as a complete block or set of replicates. Individual data observations consisted of presence/absence of roots, number of roots per cutting, and dead/alive above-ground vigour for each cutting. The effects of growing media, rooting hormones, and bottom heat on both above-ground vigour and rooting classification were analyzed using three-way logistic ANOVA (LANOVA). Due to the lack of normality in the data, the effects of the above factors on number of roots per cutting were analyzed using a Kruskal-Wallis non-parametric test. A post-hoc power analysis was performed to determine the probability of type II errors for the number of roots per cutting.

2.2.3 Rhizome cutting trials

Rhizomes from *V. membranaceum* and *V. myrtilloides* were collected from local stands of each species found within the city limits of Prince George, British Columbia, in mid-February 2008. The rhizomes were placed in cold storage at 4°C until mid-April 2008, when the *V. myrtilloides* rhizomes were removed and cut in to 8-10 cm sections containing two nodes each. Ten cuttings were then planted horizontally in growing trays and covered with 1 cm of either vermiculite or a 50:50 (by volume) peat:perlite growing medium. Half of the growing trays were kept on heating mats set to 27°C and the other half were left without bottom heat. The growing trays were covered with clear plastic domes and kept at 21710°C day/night temperatures and unsupplemented natural light conditions. Trays were placed on capillary matting and watered every 7-10 days as necessary. Final evaluation of sprouting, defined as a green shoot emerging from the substrate, categorized as present or not, was conducted 65 days after planting.

In mid-July 2008, the *V. membranaceum* rhizome cuttings, taken from the remaining stock in cold storage, were planted in the same manner as described above. Ten cuttings were planted in each growing media, however all trays were left without bottom heat. Environmental conditions and watering schedules were maintained as described above for *V. myrtilloides.* Final evaluation of sprouting was conducted 74 days after planting.

In order to get more directly comparable data for the two *Vaccinium* species, new rhizomes for both *V. membranaceum* and *V. myrtilloides* were collected from the same native stands in Prince George, B.C. in mid-October 2008. The rhizomes were prepared as described above and planted immediately in the two previously mentioned growing media. No bottom heat was applied and the environmental conditions and watering schedule were kept as previously described. Final evaluation of sprouting was conducted 95 days after planting.

Statistical analysis

Rhizome cutting experiments were set up as a randomized complete block design, with each tray serving as a complete block or set of replicates. Individual data observations consisted of presence/absence of sprouting for each cutting. The effect of rooting medium and mycorrhizal colonization on the sprouting of the rhizome cuttings was analyzed using LANOVA models.

2.2.4 Morphological comparisons

Measurements of plant height and basal stem diameter, as well as counts of number of leaves/plant were performed on 60 three month-old seedlings each of V. *membranaceum* and *V. myrtilloides,* one 10 month-old *V. membranaceum* seedling, two 10 month-old *V. myrtilloides* seedlings, 18 three month-old *V. membranaceum* rhizome cuttings, 19 three

month-old *V. myrtilloides* rhizome cuttings, one 9 month-old *V. membranaceum* rhizome cutting, and five 9 month-old *V. myrtilloides* rhizome cuttings. Heights were measured, to the nearest 1 mm, using a ruler and basal stem diameters were measured, to the nearest 0.01 mm, using digital calipers. Using the height and basal stem diameter measurements, stem volume was calculated using the formula for the area of a cylinder, $V=l/37tr^2h$, where V is the stem volume, r is the basal stem radius, and h is the stem height.

Statistical analysis

Individual data observations of height, basal stem diameter, stem volume, and number of leaves/plant for each plant were analyzed using two-way ANOVA to discern differences between plant types and species and one-way ANOVA to discern differences between species within plant types. A square-root transformation was used on the data to ensure equal variance and normal distribution of the residuals. Differences between individual plant type and species combinations were analyzed using Tukey's post-hoc test with Bonferroni adjustments.

2.3 Results

2.3.1 Seed trials

When picking berries for seed extraction, *V. membranaceum* plants were generally found in closed and partially-closed forests; whereas *V. myrtilloides* plants were found in areas with an open canopy. *Vaccinium caespitosum* plants with berries were only found in alpine and subalpine areas (Figure 2 a-c).

Figure 2. Fruiting plants and berries of *V. membranaceum, V. myrtilloides,* and *V. caespitosum.* a) *V. membranaceum* on the UNBC campus, b) *V. myrtilloides* in Mackenzie, c) *V. caespitosum* on McBride Peak, d) Representative berries of each of the three *Vaccinium* species.

Initially, the seedlings of all three species were indistinguishable from one another. When seedlings reached the 4-6 leaf stage, they began to show morphological differences between species (Figures 3 & 4).

Figure 3. *Vaccinium* seedlings from 2006 and 2007. a) Seedlings of *V. membranaceum* (centre rows) and *V. caespitosum* (outer rows) (2006). b) *V. caespitosum* seedlings at the 6-8 leaf stage (2006). c) *Vaccinium* seedlings at the 2-4 leaf stage (2007). d) *V. membranaceum* seedlings at the 6-8 leaf stage (2007).

In the 2006 trials, overall germination levels for *V. membranaceum* were 26.0 + 4.9%, overall germination levels for *V. myrtilloides* were 14.7 ± 4.9%, and overall germination levels for *V. caespitosum* were 26.7 + 6.2%. There were no statistically significant differences in germination percentage seen among seeds with different storage treatments ($F_{4,40}$= 0.49, /?=0.615) or among different species (F_{4A0}= 1-49, p=0.240) (Table 2, Figure 5). All ANOVA tables can be found in Appendix 3. A post-hoc power analysis of this trial produced a power level of 0.304, indicating only a 30.4% chance of detecting a true difference between the treatment groups at the p=0.05 level.

Figure 4. *Vaccinium* seeds and seedlings from 2007 and 2008. a) Germinating seeds of *V. myrtilloides* (2008). b) *V. membranaceum* seedlings 100 days from sowing (2008). c) *V. membranaceum* seedlings 121 days from sowing (2007). d) *V. myrtilloides* seedlings 148 days from sowing (2007).

Table 2. Mean germination percentage for *Vaccinium* seeds subjected to three different storage treatments. None of the numbers were significantly different at the p=0.05 level.

Species	**Storage Treatment**	**Mean seed germination (%)**	**Standard error**
V. membranaceum	freshly extracted	29.0	9.9
	air-dried 48 hours	32.0	9.4
	stored 1 year	17.0	6.0
V. myrtilloides	freshly extracted	20.0	9.1
	air-dried 48 hours	19.0	11.2
	stored 1 year	5.0	2.2
V. caespitosum	freshly extracted	24.0	10.2
	air-dried 48 hours	24.0	9.4
	stored 1 year	32.0	14.4

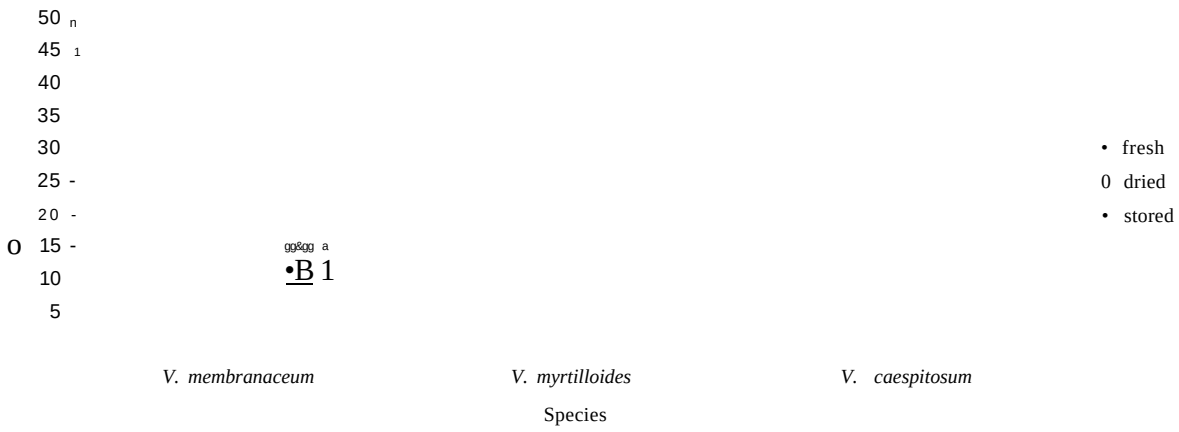

Figure 5. Mean germination percentages of V. *membranaceum, V. myrtilloides,* and V. *caespitosum* seeds than had been freshly extracted, air-dried for 48 hrs, or stored for 1 year. Error bars represent ± one standard error.

The number of seeds found within one berry, for berries collected in 2007, was significantly different among *Vaccinium* species ($F_{2,72}$= 10.55 p=0.0001) and between different provenances *(Fj,*72=2.74 /?=0.025) (Table 3). The power analysis indicated a 99.6% chance of detecting true differences between the treatment groups at the p=0.05 level. Overall, berries of *V. membranaceum* had a mean of 24.4 + 1.40 seeds/berry, berries of *V. myrtilloides* had a mean of 19.3 ± 1.18 seeds/berry, and berries of *V. caespitosum* had a mean of 16.2 + 1.07 seeds/berry.

Table 3. Mean number of seeds per berry for three *Vaccinium* species collected in the growing season of 2007. Numbers in the same species grouping followed by the same lower-case letter are not significantly different at the p=0.05 level.

Species	Provenance	Number of seeds/berry	Standard error
V. membranaceum	McBride Peak	25.6 a	2.40
	Mackenzie	19.9 a	1.75
	Teapot Mountain	27.8 a	2.56
V. myrtilloides	Aquatic Centre	20.6 a	1.91
	Coreless Park	17.0 a	2.53
	Mackenzie	20.4 a	1.59
V. caespitosum	McBride Peak	13.7 b	1.07
	Mackenzie	18.7 a	1.52

Germination percentages for the seeds of *V. membranaceum, V. myrtilloides,* and V. *caespitosum* collected in the 2007 growing season (Table 4, Figure 6) differed significantly among species (F5,24=18.79, /?=0.00001) and provenances (F5,24=5.25, p=0.006), but the number of days required to reach 50% of total germination did not differ among species (F_{5},24= 1.07, p=0.358) or provenance (F_{5i2}4=1.60, p=0.217). Overall, V *membranaceum* seeds had a mean germination percentage of 4.70 ± 1.29% and took an average of 51.5 ± 7.48 days to reach 50% of total germination, *V. myrtilloides* seeds had a mean germination percentage of 13.0 + 1.68% and took an average of 55.6 ± 2.90 days to reach 50% of total germination, and *V. caespitosum* seeds had a mean germination percentage of 17.8 + 2.08% and took an average of 44.0 +2.21 days to reach 50% of total germination. Power analysis of the total germination produced indicated a 99.9% chance of detecting true differences between treatments at the *p*=0.05 level; however, the same analysis for the number of days required to reach 50% of total germination indicated only a 40.3% chance of detecting true differences between treatments at the p=0.05 level.

Table 4. Mean germination percentage (± standard error) and mean number of days to reach 50% of total germination (+ standard error) of *Vaccinium* seeds collected over the 2007 growing season. Numbers in the same species grouping in the same column followed by the same lower-case letter are not significantly different at the p=0.05 level.

Species	**Provenance**	**Mean seed germination (%)**	**Mean # days to 50% germination**
V. membranaceum	McBride Peak	2.8 + 1.46 a	48.0 + 14.00 a
	Mackenzie	6.6+ 1.89 a	55.0 + 7.03 a
V. *myrtilloides*	Aquatic Centre	17.8+ 1.24 a	62.6 + 3.49 a
	Coreless Park	7.2 ± 1.46 b	60.2 ± 4.47 a
	Mackenzie	14.0+ 3.42 a	44.0 + 2.21 a
V. caespitosum	McBride Peak	17.8 + 2.08	44.0 + 2.21 a

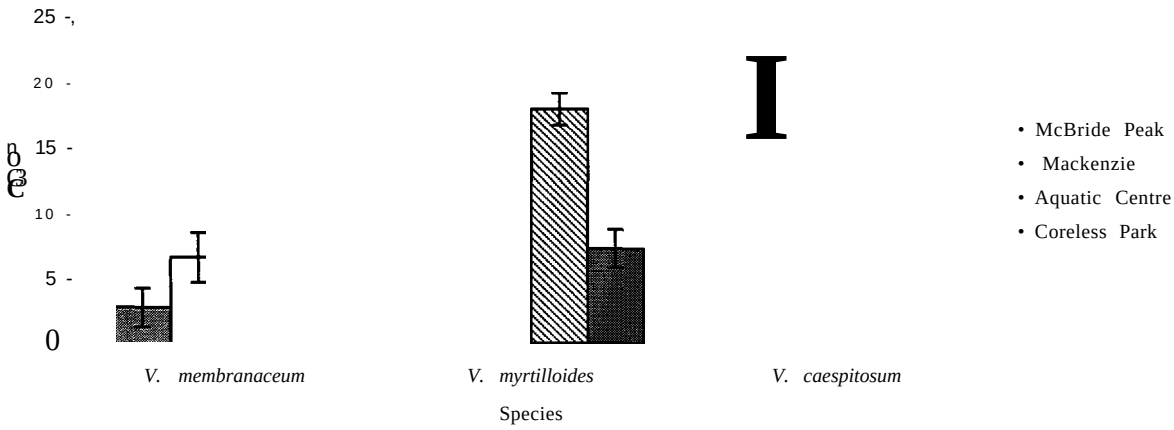

Figure 6. Mean germination percentages of *V. membranaceum, V. myrtilloides,* and *V. caespitosum* seeds collected in 2007. Error bars represent + one standard error.

Germination percentages for *V. membranaceum* and V. *myrtilloides* seeds collected during the 2008 growing season (Table 5, Figure 7) also differed significantly between species (^5,12=75.47, p=0.000002) and among provenances ($F_{5i}i_2$=7.87, p=0.002); however, the mean number of days required to reach 50% of total germination did not vary by species (^5,12=0.27, p=0.610) or provenance (F_{512}=0.85, p=0.523). Overall, *V. membranaceum* seeds had a mean germination percentage of 66.0 ± 4.24% and took an average of 46.9 ± 5.53 days to reach 50% of total germination and *V. myrtilloides* seeds had a mean germination percentage of 28.5 + 5.48% and took an average of 45.7 ± 4.73 days to reach 50% of total germination. A post-hoc power analysis of the total germination indicated a 99.9% chance of detecting true differences between treatments at the *p*=0.05 level; the same analysis for the number of days required to reach 50% of total germination indicated just an 18.4% chance of detecting true differences between treatments at the /?=0.05 level.

Table 5. Mean germination percentage (± standard error) and mean number of days to reach 50% total germination (+ standard error) of *Vaccinium* seeds collected over the 2008 growing season. Numbers in the same species grouping in the same column followed by the same lower-case letter are not significantly different at the /?=0.05 level.

Species	Provenance	Mean germination (%)	Mean # days to 50% germination
V. membranaceum	McBride Peak	52.8 ± 7.24 b	56.7 ±6.12 a
	Smithers	75.7 ±2.30 a	42.7 ± 12.03 a
	UNBC Campus	69.6 +4.17 ab	49.3 ± 11.67 a
V. myrtilloides	Coreless Park	16.0 +4.65 b	42.7 ± 8.65 a
	Crooked River	47.6 ±5.33 a	38.0 ± 9.00 a
	Kennedy Siding	22.0 ±5.39 ab	56.3 ± 4.67 a

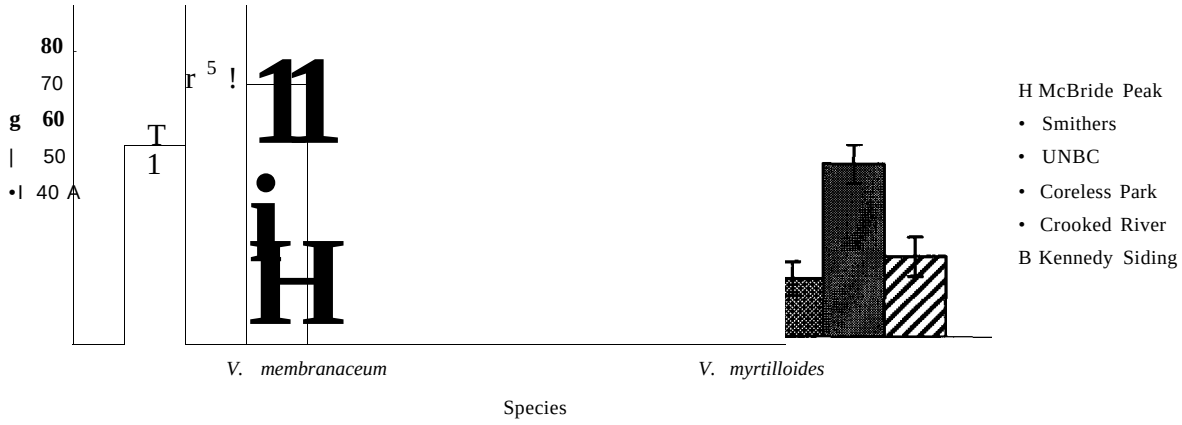

Figure 7. Mean germination percentages of *V. membranaceum* and *V. myrtilloides* seeds collected in 2008. Error bars represent + one standard error.

2.3.2 Hardwood cutting trials

None of the treatments tested impacted the development of roots in hardwood cuttings of *V. membranaceum* and *V. myrtilloides.* There were no significant differences between species *(p*=0.964), rooting substrate *(p*=0.079), rooting hormone formulation (p=0.059), or bottom heat application *(p-0.370)* (Table 6). Across all categories, the mean rooting percentages were extremely low (Figure 8a), with only six out of 240 cuttings developing roots. The mean rooting percentage for *V. membranaceum* was 2.5 ± 1.8% and the mean rooting percentage for *V. myrtilloides* was 2.5 ± 1.3%.

The above-ground vigour of the hardwood cuttings (Figure 8b) showed no significant differences between species (/?=0.169), rooting substrate *(p*=0.360), or rooting hormone formulation *(p*=0.727); however, there was a significant difference observed when bottom heat was applied to the cuttings (p=0.011) (Table 6). Hardwood cuttings placed on heating mats showed fewer individuals grouped in the "alive" above-ground vigour category than those hardwood cuttings grown without heating mats.

Kruskal-Wallis non-parametric tests indicated no significant differences for the number of roots per cuttings between different species (p=0.989), rooting substrates (p=0.100), bottom heat treatments *(p*=0.413), or hormone formulations (p=0.134). A post-hoc power analysis for the number of roots per cutting indicated a 79.7% chance of detecting true differences at the p=0.05 level.

2.3.3 Rhizome cuttings trials

The sprouting of rhizome cuttings of *V. myrtilloides* collected on February 18, 2008, and planted on April 15, 2008, showed no significant differences between rhizomes planted in vermiculite and those planted in a 50:50 peat/perlite (by volume) substrate (p=1.00); however, those rhizome cuttings placed on heating mats showed significantly lower sprouting percentages than those without any bottom heat application *(p*=6.03 x 10^{-9}). No rhizome cuttings placed on heating mats showed any sprouting (Table 7); consequently all subsequent trials were conducted without bottom heat application. Overall, the cuttings in this trial had a sprouting level of 80%. The sprouting of rhizome cuttings of *V. membranaceum* collected on February 18, 2008 and planted on July 15, 2008 showed no significant differences between rhizomes planted in the different growing media tested *(p*=0.0835). Overall, the cuttings in this trial had a sprouting level of 20%.

Rhizome cuttings of *V. membranaceum* and *V. myrtilloides* collected and planted on October 11, 2008 (Figure 8 c-d) showed no significant differences in sprouting between the two species *(p=0.252)* or the two different growing media mentioned above (p-0.700). Overall, the V. *myrtilloides* cuttings in this trial had a sprouting level of 85% and the *V. membranaceum* cuttings had a sprouting level of 70%.

Table 6. Percentage of *Vaccinium* hardwood cuttings showing presence of roots and above-ground vigour and mean number of roots per cutting (+ standard error) grouped by rooting substrate, application of bottom heat, and hormone formulation.

Species	Rooting substrate[1]	Bottom heat	Hormone formulation[2]	Cuttings showing presence of roots (%)	Cuttings showing above-ground vigour (%)	Mean# roots/cutting
V. membranaceum	peat/perlite	+	IBA	10.0	40.0	0.10 + 0.10
			NAA	0	20.0	0
			IBA+NAA	0	0	0
	peat/perlite	-	IBA	20.0	50.0	0.30 + 0.21
			NAA	0	20.0	0
			IBA+NAA	0	50.0	0
	rockwool	+	IBA	0	10.0	0
			NAA	0	10.0	0
			IBA+NAA	0	0	0
	rockwool	-	IBA	0	0	0
			NAA	0	40.0	0
			IBA+NAA	0	30.0	0
V. myrtilloides	peat/perlite	+	IBA	0	10.0	0
			NAA	0	10.0	0
			IBA+NAA	10.0	10.0	0.30 + 0.30
	peat/perlite	-	IBA	10.0	20.0	0.10 ±0.10
			NAA	0	10.0	0
			IBA+NAA	0	10.0	0
	rockwool	+	IBA	0	20.0	0
			NAA	0	10.0	0
			IBA+NAA	0	10.0	0
	rockwool		IBA	0	20.0	0
			NAA	0	30.0	0
			IBA+NAA	10.0	20.0	0.20 + 0.20

rooting substrates included a 50:50 peat/perlite mixture (by volume) and commercial grade rockwool
[2]hormone formulations included 20000ppm IBA, lOOOOppm NAA, and a combination of 20000ppm IBA + lOOOOppm NAA

Table 7. Percentage of *Vaccinium* rhizome cuttings showing sprouting grouped by collection and planting date, growing media, and bottom heat application.

Collection date	Planting date	Species	Growing medium[1]	Bottom heat	Cuttings showing sprouting (%)
February 18, 2008	April 15, 2008	*V. myrtilloides*	peat/perlite	+	0.0
				-	80.0
			vermiculite	+	0.0
				-	80.0
February 18, 2008	May 15, 2008	V. *membranaceum*	peat/perlite	n/a	0.0
			vermiculite	n/a	20.0
October 11, 2008	October 11, 2008	*V. myrtilloides*	peat/perlite	n/a	90.0
			vermiculite	n/a	80.0
		V. membranaceum	peat/perlite	n/a	70.0
			vermiculite	n/a	70.0

growing media included a 50:50 peat/perlite mixture (by volume) and commercial grade vermiculite

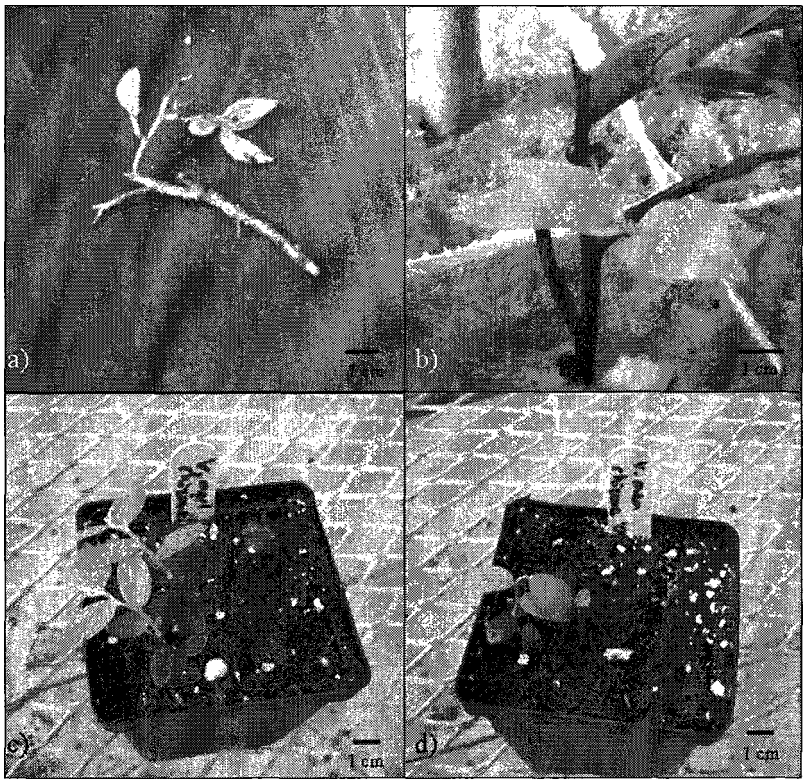

Figure 8. Hardwood cuttings and rhizome cuttings of *V. membranaceum* and *V. myrtilloides.* a) *V. myrtilloides* hardwood cutting showing one root after 62 days, b) *V. membranaceum* hardwood cutting growing in rockwool after 50 days, c) *V. myrtilloides* rhizome cutting 153 days since planting, d) *V. membranaceum* rhizome cutting 153 days since planting.

2.3.4 Morphological comparisons

The height measurements of *Vaccinium* plants differed significantly among 3 month-old seedlings, 10 month-old seedlings, 3 month-old rhizome cuttings, and 9 month-old rhizome cuttings (F4,i6i=l 11.66, *p*- < 2.2 x 10^{-16}). Nine month-old rhizome cuttings had the tallest plants, with a maximum height of 128 mm, followed by 10 month-old seedlings, 3 month-old rhizome cuttings, and 3 month-old seedlings (Figure 9, Table 8).

The basal stem diameter of *Vaccinium* plants also differed among different plant types (F_{4i}i6i=327.01, *p*= < 2.2 x 10^{-16}). The largest basal stem diameter was seen in 9 month-old rhizome cuttings, with a maximum of 2 mm, followed by 10 month-old seedlings, 3 month-old rhizome cuttings, and 3 month-old seedlings (Figure 9, Table 8).

The calculated stem volume of *Vaccinium* plants differed among plant types as well (F_4,161=144.90, *p*= < 2.2 x 10^{-16}). The 9 month-old rhizome cuttings had the largest stem volume, with a maximum of 181 mm^3, followed by the 10 month-old seedlings, 3 month-old rhizome cuttings, and 3 month-old seedlings (Figure 9, Table 8).

The number of leaves/plant of *Vaccinium* plants also differed among 3 month-old seedlings, 10 month-old seedlings, 3 month-old rhizome cuttings, and 9 month-old rhizome cuttings (F4,i6i=158.37, *p*- < 2.2 x 10^{-16}). Ten month-old seedlings had the most leaves/plant, with a maximum of 39 leaves/plant, followed by 9 month-old rhizome cuttings, 3 month-old seedlings, and 3 month-old rhizome cuttings (Figure 9, Table 8).

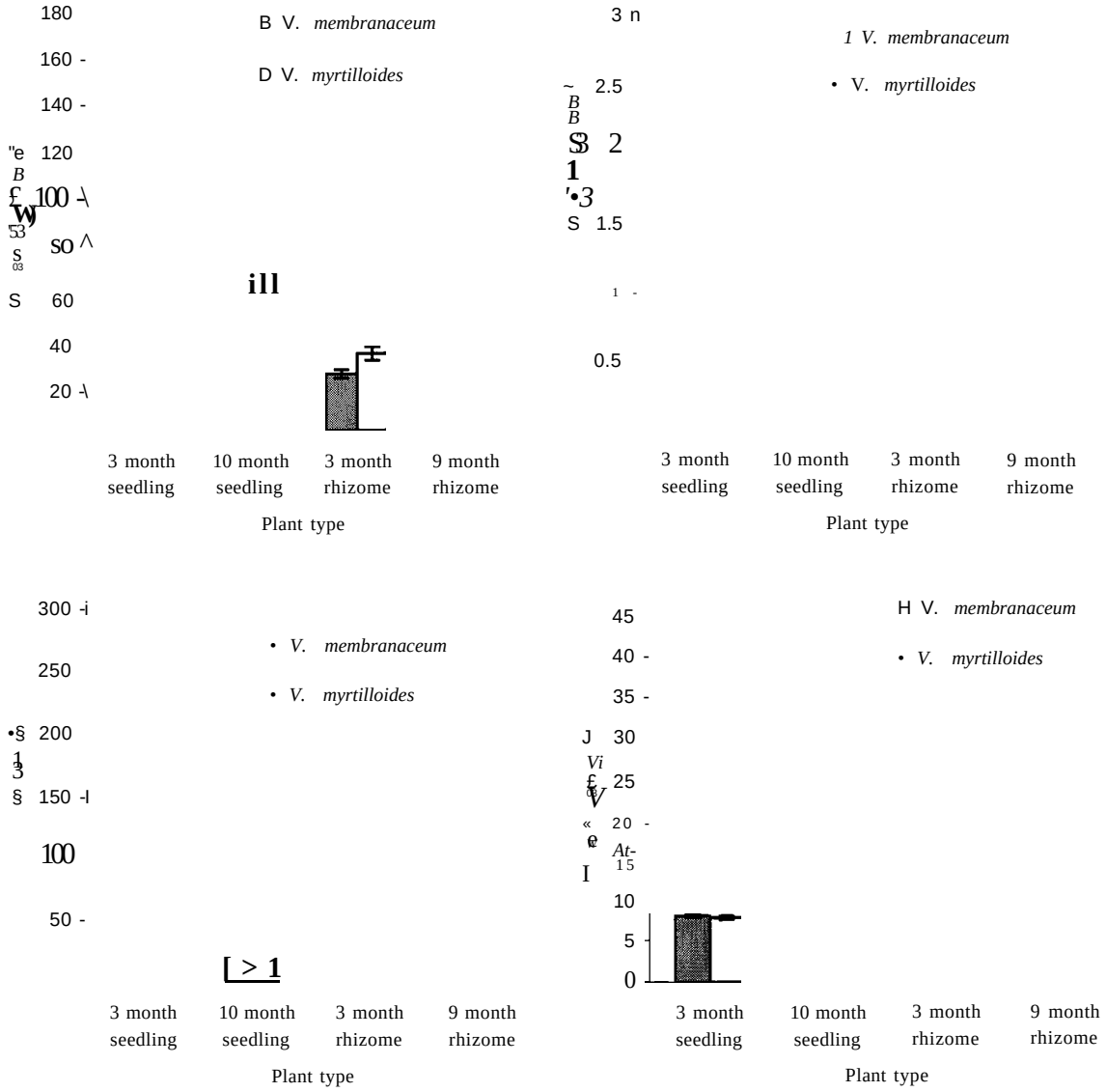

Figure 9. Mean height, basal stem diameter, stem volume, and number of leaves/plant of 3 month-old seedlings, 10 month-old seedlings, 3 month-old rhizome cuttings, and 9 month-old rhizome cuttings of *V. membranaceum.* and *V. myrtilloides.*

Within the 3 month-old seedlings, *V. membranaceum* plants had significantly taller plants ($F_{1,18}$=38.72, p=*1.12* x 10^{-9}) as well as larger basal stem diameters than *V. myrtilloides* plants ($F_{1,18}$=55.95, p= 1.44 x 10^{-11}). The number of leaves/plant did not vary between the two species ($F_{1,18}$=0.63, p=0.428). Within the 3 month-old rhizome cuttings, *V.*

membranaceum plants had larger basal stem diameters (Fi_{j35}=4.82, p=0.035), were shorter (Fi,35=5.42, *p*=0.026) with fewer leaves/plant (F_{U5}=19.87, p=8.164 x 10"^{5}) than V. *myrtilloides* plants. Comparisons between the two species of 10 month-old seedlings and 9 month-old rhizome cuttings were not conducted due to inadequate sample sizes.

Table 8. Height, basal stem diameter, stem volume, and number of leaves/plant (± standard error, where applicable) for *V. membranaceum* and *V. myrtilloides* plants as 3 month seedlings, 10 month seedlings, 3 month rhizome cuttings, and 9 month rhizome cuttings. Numbers for the same plant type in the same column followed by the same lower-case letter are not significantly different at the p=0.05 level.

Plant type	Species	Mean height (mm)	Mean basal stem diameter (mm)	Mean stem volume (mm^3)	Mean# leaves/plant	n
3 month	*V.membranaceum*	18.38 ±0.58 a	0.41 ±0.01 a	0.85 ±0.05 a	7.98 ±0.21 a	60
seedling	*V. myrtilloides*	13.35 ± 0.58 b	0.32 ±0.01 b	0.38 ± 0.03 b	7.75 ± 0.22 a	60
10 month	*V. membranaceum*	36.00 a	1.83 a	31.56 a	35.00 a	1
seedling	*V. myrtilloides*	70.00 ± 9.00 a	1.01 ±0.04 a	18.70 ±3.67 a	38.50 ±3.50 a	2
3 month	*V.membranaceum*	24.11 ± 1.77 b	0.91 ±0.06 a	6.14 ± 1.50 a	4.50 +0.37 b	18
rhizome	*V. myrtilloides*	32.89 ± 2.90 a	0.76 ± 0.03 b	5.33 ±0.72 a	8.32 ±0.81 a	19
9 month	*V. membranaceum*	30.00 a	0.58 b	2.64 b	7.00 b	1
rhizome	*V. myrtilloides*	128.40 ± 39.89 a	2.15 ±0.24 a	180.91 ±69.01 a	30.40 ± 3.75 a	5

2.4 Discussion

2.4.1 Seed trials

None of our seed storage treatments impacted total germination percentages (Table 2), however, sample size and within and between treatment variability indicated there was only a 30% chance of detecting true differences given our experimental set-up. In contrast to our study, *V. membranaceum* seeds from Idaho that had been air dried for 7 days had reduced germination compared to those stored for 7 years, 1 year, or freshly extracted (Shafii and Barney 2001). Previously, air dried *V. membranaceum* seeds had shown a slight, but not statistically significant, decrease in total germination percentage (Barney 1996). The

differences between these results and the ones obtained in the present study could be related to the moisture content of the seeds. Low moisture contents, usually approximately 10%, are desirable for the long term storage of seeds, because they inhibit the growth of insects and diseases (Dirr and Heuser 1987, Banerjee *et al.* 2001). In order for germination to occur, however, seeds must imbibe water to increase their moisture content (Rose *et al.* 1998). The air drying period in the present study was only 2 days, as opposed to 7 days in the Idaho study. The shorter time period would have allowed less time for desiccation of the seeds prior to sowing, leading to higher moisture contents and higher germination rates. Seeds of *V. myrtilloides* that had been frozen, refrigerated at 1°C, and freshly extracted showed no differences in total germination percentage when tested in Nova Scotia (Vander Kloet and Hall 1981), supporting the findings of this study. *Vaccinium corymbosum* seeds stored for 12 years have been shown to germinate up to 86.2%, and V. *asheii* seeds stored for 9 years have been shown to germinate up to 59.0% (Darrow and Scott 1954). This study did not look at time periods as great as those described above, but the results suggest that *V. membranaceum*, *V. myrtilloides*, and *V. caespitosum* seeds may also have the ability to retain germination potential when stored at proper temperatures and moisture content levels.

The overall total germination rates of the *Vaccinium* seeds, especially those for *V. membranaceum*, were considerably higher in the 2008 germination trials compared to the 2006 and 2007 germination trials. This may be due to natural variability in the seed lots, but also could be tied to the methods used to determine germination. In 2006 and 2007, seeds were surface sown on a peat-sand mixture and monitored in the greenhouse for germination. In 2008, seeds were placed on moistened filter paper inside sealed Petri dishes in the laboratory and monitored for germination. The absence of any potting media in the 2008

trials allowed for the early observation of very small germinating seeds. These seeds were included in the germination counts; however, in previous years germinants of this size may not have been visible through the potting media. When germinants reached a size that could be observed on the potting media it is possible that previously germinated seeds had died. In light of these methodological differences, it may be useful to view the germination percentages reported for 2006 and 2007 as seedling emergence percentages that may be lower than true germination percentages, but more representative of field conditions.

In the 2006 and 2008 trials, *Vaccinium myrtilloides* seeds had the lowest germination percentages of the three species studied, averaging 15% and 29%, respectively. *Vaccinium myrtilloides* has been reported as being variable in its germination behavior, with levels of 11-51% reported in eastern Canada (Vander Kloet and Hall 1981). The germination methods used to obtain these numbers were not reported, which makes it difficult to use them for comparison purposes. Our results do fall within the same range, regardless of which germination method was used, suggesting that the germination behaviour of *V. myrtilloides* seed is similar for plants in the eastern and western parts of Canada.

Maximum germination levels of *V. membranaceum* seeds have been reported as high as 80% in the southern half of British Columbia (McLean 1967), and 70%, 79%, and 74% in Idaho (Barney *et al.* 2001; Barney 2003; Shafii and Barney 2001). All of the Idaho studies used the Petri plate method of germination, whereas the methods used by McLean (1967) are unclear. The results found in our study, with germination ranging from 5% to 66%, consistently fall lower than these reported maxima. Germination performance of *V. membranaceum* can be improved by surface sterilization or the use of fungicides (Barney 2003), but neither of these methods was used in the present study. *V. membranaceum* seeds

have also been shown, using lab-based Petri plate methods, to need exposure to light in order to reach their maximum germination potential (Haeussler *et al.* 1990; Barney 2003). This has also been shown to be true for *V. myrtillus, V. uliginosum,* and *V. vitis-idaea* seeds in Sweden (Baskin *et al.* 2000) Seeds from the 2006 and 2007 germination trials sown on potting media may have had some light blocked by the coarse nature of the peat, although they were surface sown. This may help account for some of the lower numbers observed.

Germination levels for *V. caespitosum* have been reported as high as 96% (McLean 1967), which is higher than our results of 18% and 27%. Very little literature specifically concerning *V. caespitosum* has been reported. *Vaccinium caespitosum* is found in section *Myrtillus,* which is also the same section that contains *V. membranaceum* (Vander Kloet 1988); therefore the reasons for the lower germination percentages in our study may be the same as those proposed above for V. *membranaceum* seeds. Our seeds also came from high elevation sites (see Appendix 1), which may have inherently lower germination potential than those found in lower elevation areas.

Although the germination percentages found for *V. membranaceum, V. myrtilloides,* and *V. caespitosum* in this study varied between seed sets and treatments, the number of days required to reach 50% of maximum germination stayed relatively constant at 44-55 days. This suggests that the speed of germination may be less affected by external factors than germination percentages. The number of days required to reach 50% of maximum germination for *V. membranaceum* seeds has been reported between 16 and 39 days (Barney *et al.* 2001; Shafii and Barney 2001). These numbers are lower than the ones reported in this study. This may be due to regional differences, as the reported studies were conducted using berries from Idaho, whereas this study was conducted using berries from northern British

Columbia. Seeds from colder climates may be genetically predisposed to germinate more slowly to accommodate later growing seasons. If the seeds germinated faster, the resulting seedlings might face inhospitable growing conditions.

The number of seeds per berry varied between the three species studied. These differences can be explained by the size of the berries (Figure 2d). *Vaccinium membranaceum* has the largest berries of these three species, which could be expected to hold more seeds, which are all approximately the same size, than the smaller *V. myrtilloides* or *V. caespitosum* berries. In the same manner, *V. myrtilloides* berries are larger than *V. caespitosum* berries and therefore could be expected to hold more seeds. *Vaccinium myrtilloides* has been reported has having 9-31 seeds/berry (Vander Kloet and Hall 1981). Other *Vaccinium* species, including *V. angustifolium, V. boreale, V. vitis-idaea, V. oxycoccus, V. macrocarpum,* and *V. uliginosum,* have had numbers of 8-25 seeds/berry reported (Vander Kloet and Hill 2000). The results presented here fall within these ranges and are not likely uncommon.

2.4.2 Hardwood cutting trials

The hardwood cuttings harvested in winter of 2007 of both *V. membranaceum* and *V. myrtilloides* had lower survival rates than semi-hardwood cuttings of *V. membranaceum* in Montana, which rooted at a level of 7% (Evans *et al.* 2001). The results in this study are much lower than those reported from Montana, which may be explained by timing. Hardwood cuttings are taken over the winter months, after plants have lost their leaves. Semi-hardwood cuttings are taken in late summer or early fall, after growth has finished but before leaves are lost. In comparison, softwood cuttings are taken from emerging shoots of growing plants (Dirr and Heuser 1987). While *Vaccinium* hardwood cuttings do not show

high survival levels, softwood cuttings of *V. corymbosum* have been reported to root at levels up to 80% (Dirr and Heuser 1987) and *V. angustifolium* has been shown to root up to levels of 99% when taken from softwood cuttings (Kender 1967b). These differences may relate to the timing of the cuttings, but also may be due to horticultural selection of plants that are easy to propagate using cuttings. Both *V. corymbosum* and *V. angustifolium* are commercially grown and managed crops which have likely been subjected to selection for easy propagation characteristics.

Different hormone formulations did not have any significant effect on the rooting, above-ground vigour, or number of roots per cutting of the hardwood cuttings. Although not statistically significant, it can be noted that all of the rooted *V. membranaceum* cuttings in our study were found in the IBA treatment while all of the rooted *V. myrtilloides* cuttings were found in either the IBA treatment or the IBA + NAA treatment (Table 6). Studies using solely NAA formulations as rooting hormones are rare, suggesting that perhaps NAA is not a suitable rooting hormone on its own. In contrast to our study, *V. vitis-idaea* softwood cuttings have been shown to show improved growth with IBA application up to a concentration of 5000 ppm (Gorecka 1979; Debnath 2006). Easier to root cuttings, such as *Populus balsamifera* L., have been shown to have no response to the addition of IBA (DesRochers *et al.* 2004). Given these findings for different species, it is possible that the optimal levels of IBA and NAA for *V. membranaceum* or *V. myrtilloides* was fall outside of the range tested in this study. The high concentrations of IBA and NAA used may have masked any differences that occur between these formulations, or even proved detrimental to the rooting of these species. The absence of a control group also makes the results difficult to interpret.

The rooting media used also did not have any significant effects on the rooting, above-ground vigour, or number of roots per cutting of *V. membranaceum* or *V. myrtilloides* cuttings (Table 6). A proper rooting medium for any type of cuttings should provide oxygen space, water-holding capacity, and structural support (Dobson 2000). As both of the rooting media used in this study fulfill these requirements, the lack of any significant differences is not unexpected. Peat/perlite mixtures have been shown to increase rooting percentages in *Rosa dumalis* Bechst. hardwood cuttings when compared to those grown in sawdust and peat/sawdust mixtures (Erci§li *et al.* 2005). Perlite adds porosity to a peat mixture, allowing more oxygen to reach the growing portions of the cuttings and promoting drainage. Perlite is also used in the rooting of *Arctostaphylos* hardwood cuttings because it is light and will not damage delicate newly-formed roots (Borland and Bone 2007).

The presence of bottom heat did not significantly affect the rooting or number of roots per cutting of *V. membranaceum* or *V. myrtilloides* hardwood cuttings; however, it had a negative influence on the above-ground vigour of the cuttings (Table 6). Bottom heat has been found to be beneficial to some species of hardwood cuttings (Dirr and Heuser 1987); however, this does not appear to be the case with *Vaccinium.* The heating mats may have caused an increase in the air temperature around the cuttings, leading to increased evapotranspiration from the leaves that had sprouted from the cuttings while in the greenhouse (Taiz and Zeiger 2002) and increased water stress for the cuttings in the bottom heat treatment, which may have led to the decrease in above-ground vigour. The increased air temperature may also have provided a more hospitable environment for plant pathogens, although no visible pathogens were observed during the trials.

2.4.3 Rhizome cutting trials

The sprouting of rhizome cuttings of *V. membranaceum* and *V. myrtilloides* was not significantly influenced by the growing media in which they were planted (Table 7). This observation held true for all of the different rhizome trials conducted. The requirements for rhizome cutting media are the same as those for hardwood cuttings, namely the ability to hold water, oxygen, and provide structural support (Dobson 2000). As rhizome cuttings are planted beneath the surface of the media, instead of being placed upright, the media requires less structural support than it might if shoot cuttings were being planted. Vermiculite is recommended as a medium for V. *angustifolium* rhizome cuttings by Dirr and Heuser (1987), however it would not have the ability to support upright shoot cuttings.

The first rhizome trials with *V. myrtilloides* found that bottom heat negatively affected the sprouting of the cuttings (Table 7): none of the cuttings placed on the heating mats sprouted. The additional heat from the heating mats likely caused the growing media to lose water faster than those trays without heating mats. This would have caused the rhizome cuttings to desiccate and die, leading to no sprouting observed. Further trials were conducted without the bottom heat variable to avoid this outcome.

The sprouting percentages of the *Vaccinium* rhizomes in the second trial varied from 20%-90%. The lower 20% figure was obtained for *V. membranaceum* rhizome cuttings that had been kept in cold storage for five months. Spending months in cold storage had reduced the carbohydrate reserves, and hence the sprouting potential for these rhizome cuttings (Dirr and Heuser 1987). Rhizome cuttings of both *Vaccinium* species that were planted immediately after being collected showed higher sprouting percentages. *V. angustifolium* rhizome cuttings have been reported to sprout at levels up to 66% (Kender 1969). Rose *et al.*

(1998) found that *V. membranaceum* could be rooted from rhizome cuttings taken in early spring; however no success levels are indicated. Given these reports, the results presented here appear to represent a high level of success with *Vaccinium* rhizome cuttings.

2.4.4 Morphological comparisons

When comparing the morphological characteristics of different forms of *Vaccinium* plants, it is useful to compare the plants of the same age category. Nine month-old rhizome cutting plants of *V. membranaceum* and *V. myrtilloides* were taller and had larger basal stem diameters, and consequently larger stem volumes, than 10 month-old seedlings of the same species; however, they had fewer leaves per plant. Similarly, when looking at the younger plant forms, 3 month-old rhizome cuttings were taller, had larger basal stem diameters, larger stem volumes, and had fewer leaves/plant than 3 month-old seedlings (Table 8). Three month-old plants would logically be expected to be smaller than nine or ten-month old plants. Rhizome cuttings were consistently taller and had larger basal stem diameters than seedlings of comparable age. Rhizome cuttings grow faster than seedlings because they have access to carbohydrates from the parent plant stored in the rhizome. These energy supplies would allow the plants to grow taller and thicker faster than seedlings, which would have to procure their carbohydrates through photosynthesis. Ten months would not appear to be enough time to overcome this difference. Seedlings of both age groups had consistently more leaves per plant than plants from rhizome cuttings. As mentioned previously, seedlings are obtaining their energy through photosynthesis and could be expected to produce more leaves to capture sunlight compared to plants from rhizome cuttings. The differences in the number of leaves per plant could also be attributed to the growth form of the plants. Rhizome cuttings produce plants growing in the adult form, whereas seedlings may take

several years to achieve a mature growth form. Mature plants usually have fewer, but larger, leaves compared to younger plants.

2.5 Conclusions and Recommendations

In this study, seeds of *V. membranaceum, V. myrtilloides,* and *V. caespitosum,* and hardwood cuttings and rhizome cuttings of *V. membranaceum* and *V. myrtilloides* were tested under a variety of conditions. Seeds of these three species were found to vary in total germination based on species and collection location, but not with different seed storage times. Future studies on seed storage could include more replications to increase the power of statistical analysis, more storage types, including freezing, and measures of moisture content. Further studies on seed germination could also include pre-sowing treatments, such as stratification, as the influences of these factors do not appear to be well understood for these three *Vaccinium* species.

Hardwood cuttings of *V. membranaceum* and *V. myrtilloides* rooted very poorly and were not influenced by rooting media or hormone formulation. Bottom heat appeared to be detrimental to above-ground vigour. Further studies using hardwood cuttings could attempt to determine the optimal rooting hormone strength for these species, using a control group and a wider range of hormone concentrations to see if the rooting hormones are having any effect at all, or experimenting with the timing of the collection of cuttings. Trials using softwood cuttings should also be undertaken for comparison purposes. Those propagating *V. membranaceum* or *V. myrtilloides* using hardwood cuttings should avoid using bottom heat, as it would appear to have adverse effects on the cuttings. In general, propagation from hardwood cuttings is not as effective as propagation from seed or rhizomes.

Sprouting of rhizome cuttings of *V. membranaceum* and *V. myrtilloides* was not influenced by the growing medium in which they were planted. The presence of bottom heat reduced the sprouting of the rhizome cuttings. Further studies of rhizome propagation could include increased types of growing media, as well as experimenting with the timing of rhizome collection and storage times. Those propagating either of these species using rhizome cuttings should avoid using bottom heat because of decreased sprouting.

Mass-producing either *V. membranaceum* or *V. myrtilloides* plants would be easiest to do using seedlings. Low germination rates can be overcome by over-sowing for the number of final plants desired, since very little effort is required to collect large quantities of seed. Seeds should be collected from a variety of locations, or locations closest to where the seedlings are intended to be planted, to ensure a higher likelihood of robust seedlings. If plants identical to the parent plant were desired, rhizome cuttings would be the best method of propagation. This would also be the preferred method of propagation if plants were required in a short period of time, as seedlings grow quite slowly. The collection of rhizome cuttings can be quite damaging to the parent plant, if done without due care and consideration; therefore this method should only be used if parent plants are available in-stock or natural areas that have been scheduled for disturbance, such as road building or pipe line construction, can be identified. This method should not be used in natural areas that are unlikely to be otherwise disturbed, unless collectors are properly trained. The use of hardwood cuttings in mass-production of *Vaccinium* plants is not recommended at any time considering the low success rates found. Further studies of hardwood and rhizome cutting propagation of *V. caespitosum* would be required before any recommendations could be made.

3.0 Influences of fungal inoculation on *V. membranaceum* and *V. myrtilloides* seedling survival and growth.

3.1 Introduction

Ericoid mycorrhizal relationships benefit ericaceous plants, including *Vaccinium* spp., by allowing them to access nutrients that would otherwise be unavailable. The mycorrhizal fungi involved in these relationships appear to be ubiquitous in natural environments, but they are usually absent from potting mixes used in greenhouses. This is most likely because they can be killed by the water, fertilizers, pesticides, and fungicides used in most horticultural systems (Acree and Appleton 2000). Commercial inoculants are available for some mycorrhizal fungi; however they are mostly developed for the more common arbuscular mycorrhizal species (Corkidi *et al.* 2008). The addition of mycorrhizal inoculant has been found to be beneficial to the rooting of several species of ericaceous shoot cuttings (Scagel 2004, 2005). The inoculation of several cultivated *Vaccinium* species with laboratory cultures of ericoid mycorrhizal fungi has been shown to increase fruit yield (Powell and Bates 1981) and plant dry weight (Powell 1982; Scagel 2005); however the influences of ericoid mycorrhizal inoculation on the growth and survival of *Vaccinium* species native to western North America has not been examined.

This study was designed to look at factors influencing the performance of *Vaccinium* seedlings when they are moved from greenhouse to outdoor conditions. Two outdoor field trials and one indoor greenhouse study were set up to determine the influence of mycorrhizal inoculation and soil amendments on the survival and growth of *V. membranaceum* and *V. myrtilloides* seedlings. These trials were undertaken with the hypothesis that mycorrhizal inoculation would benefit the survival and growth of these two *Vaccinium* species in a

manner similar to a forest floor soil amendment, which was presumed to contain adequate natural inoculum.

3.2 Methods and Materials

3.2.1 Field planting trials

Three provenances of *V. membranaceum* berries, three provenances of V. *myrtilloides* berries, and two provenances of *V. caespitosum* berries were collected from north-central British Columbia during the summer of 2007. Seed collection locations and dates for all trials are given in Appendix 1. Berries were stored at 4°C until late October 2007 when the seeds were extracted in the manner described in the previous chapter. Seeds were allowed to air dry for 24 hrs, and stored at 4°C for 25 days prior to sowing. The seeds were then surface sown on to a 50:50 peat:sand growing medium and kept under greenhouse conditions with a 21°/10°C day/night temperature and 15 hour photoperiod. Seed trays were covered with clear plastic domes, placed on capillary matting, and watered every 7-10 days, as necessary, and a 20-20-20 N-P-K fertilizer was applied every three weeks mixed into the irrigation water at a level of 50 ppm. Trays were placed on greenhouse benches in a random order and re-randomized every three weeks. After 108 days, the seedlings were transplanted into larger growing cells containing the same growing medium. The clear plastic domes were removed from the growing trays at the time of transplanting. At this point, although they had germinated, there were no surviving *V. caespitosum* seedlings, therefore they are absent from subsequent trials.

One hundred and eighty-two days after sowing, when seedlings were at the 4-10 leaf stage, seedlings were inoculated with one of three ericoid mycorrhizal fungi, *Rhizoscyphus ericae* (D.J. Read) W.Y. Zhuang & Korf, *Oidiodendron maius* Barron, or *Meliniomyces* sp.,

or a control solution (Figure 10 a-c). Fungal cultures were obtained from previously DNA-sequenced species isolated from *V. membranaceum* roots growing in the McBride, British Columbia (B.C.) area and grown in the laboratory in a manner modified from Litten *et al.* (1992), Yang *et al.* (2002), and Scagel (2004, 2005). The fungal cultures were grown on a potato dextrose agar (PDA) solution prepared at 20% of the recommended label strength. These fungal cultures were then diluted 2:1 with more 20% PDA solution and macerated in a blender for one minute to obtain a useable inoculant (Figure lOd). Three mL of inoculant was pipetted on to the surface of the growing media at the base of each seedling. Control group seedlings were inoculated with a sterile 20% PDA solution in order to keep the nutrient levels equal between the treatments. Excess inoculant was brought back to the laboratory where the number of hyphal clusters/mL in each solution was measured using a hemacytometer.

One week after the seedlings were inoculated, all growing trays were moved to a sheltered outdoor location in order to allow the plants to become acclimatized to outdoor conditions (Figure 12a-b). On June 19 and 20, 2008, 29 and 30 days after inoculation, respectively, 1440 seedlings were transplanted to three field sites on the University of Northern British Columbia (UNBC) campus in Prince George, B.C. (Table 9).

Field sites were scoured to a depth of five centimeters using a Thomas® skid steer loader to remove any existing vegetation and break up the soil. The loosened soil was then added back to the plots and manually mixed with a Garden Claw® until there was a loose rooting zone of 10 cm into which the seedlings were planted. Sixty seedlings (2 species x 3 provenances x 10 replicates) were planted 20 cm apart into each of eight 1.4 m x 1.4 m plots

Figure 10. Fungal cultures growing on PDA and prepared inoculant. a) *Rhizoscyphus ericae.* b) *Oidiodendron maius.* c) *Meliniomyces* sp. d) Prepared inoculum of *Oidiodendron maius.*

Table 9. Field site location data for UNBC campus, Prince George, B.C.

Location	Latitude/ Longitude	Elevation (m)	Distance to A (m)	Distance to B (m)	Distance to C (m)
A - Snow storage area north of Teaching and Learning Building	53°53'47.10" N 122°48'51.71"W	713	n/a	760	880
B - Research area west of residence buildings	53°53'23.06" N 122°49'03.23"W	713	760	n/a	400
C - Future soccer field south of Northern Sport Centre	53°53'20.14" N 122°48'36.80"W	713	880	400	n/a

in a randomly assigned order (Figure 11). Half of the plots in each site were amended with the equivalent of a 10-cm thickness of local forest floor material (litter, fermentation, and humus layers, LFH, including some rotting wood, collected from locations with native *Vaccinium* populations), while the other half were left unamended (Figure 12 c-d). Each plot contained one of the four inoculation treatments and plots were separated by 30-cm buffer zones to decrease the chances of cross-contamination of root microbes. Excess seedlings were planted around the perimeter of each plot to reduce edge effects on the trial seedlings. Field plots were watered every 1-2 days, or as necessary depending on natural precipitation levels. Survival of all seedlings was assessed every 2-3 weeks throughout the growing season.

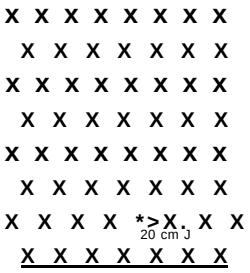

Figure 11. Schematic representation of field site layout with an entire site on the left and a cut-out of individual seedling locations in each plot shown on the right.

Because many transplanted seedlings in the initial field trial died during an unusual hot spell immediately after outplanting, a supplemental trial of *V. membranaceum* and *V. myrtilloides* seedlings was established on the UNBC campus in mid-August, 2008. Fifty-two

uninoculated *V. myrtilloides* seedlings and 12 uninoculated *V. membranaceum* seedlings were planted into each of two field plots. The plots were prepared as described above, with one field plot amended with local forest floor material and the second left without amendments. The plots were watered daily, or as necessary, given local precipitation patterns.

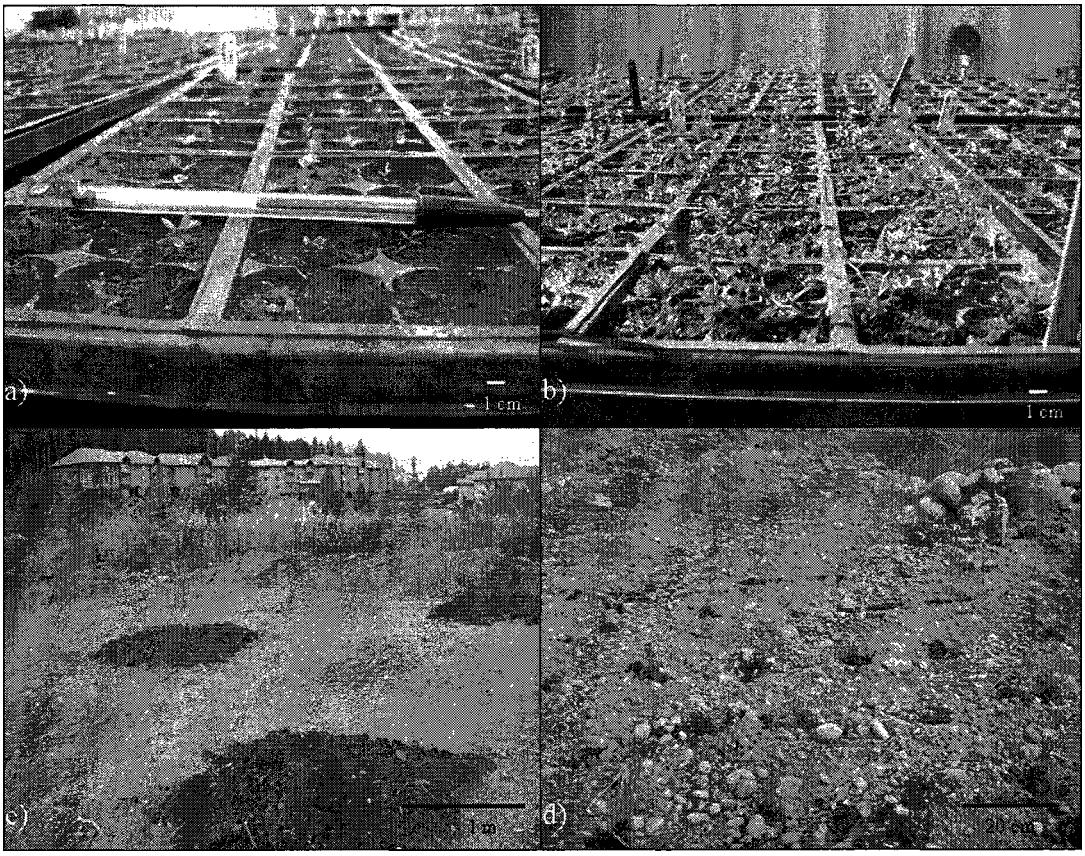

Figure 12. Seedlings prior to transplanting and field site layout, a) *Vaccinium* seedlings growing in greenhouse conditions, b) *Vaccinium* seedlings moved outdoors for hardening off. c) One field site showing amended and control (unamended) plots, d) Unamended (control) plot showing placement of individual seedlings.

Mycorrhizal and Growth Analysis

At the end of the growing season, 110 days after the initial trial was planted and 60 days after the supplemental trial was planted, all surviving seedlings were harvested and

placed in cold storage at 4°C. The root systems of the seedlings were washed clean of any debris and small samples of hair roots were taken from each plant prior to drying. The root samples were stored in 50% ethanol at room temperature. Samples were washed three times in sterile water and cleared in 5% H2O2 at 60°C for 12 hours. Root samples were then rinsed another three times in sterile water and stained in 1:1 lactoglycerol and 0.03% chlorazol black E at 60°C for four hours (Brundrett and Kendrick 1990). Roots were then mounted on slides with 1:1 lactoglycerol and examined using light microscopy at 400X-1000X magnification for the presence of mycorrhizal colonization. Colonization was scored as presence or absence of hyphal coils in the epidermal cells. For comparison purposes, root samples were taken from five *V. membranaceum* and five *V. myrtilloides* plants grown from rhizome cuttings and processed and examined in a similar manner.

After root samples were collected, seedlings were dried for 48 hrs at 70°C, separated into above- and below-ground portions, and weighed to the nearest 0.0001 g using a Sartorius Micro® electronic balance.

Soil Analysis

Composite soil samples were collected from the amended and unamended plots at all three field sites from the initial trials, as well as samples of the peat.sand media used for growing seedlings in the greenhouse. These samples were air dried for 48 hours and run through a 2 mm sieve. Soil pH was then measured in the manner described by Hendershot *et al.* (2008). Ten g of the mineral soils, or 2 g of the organic media, were added to 20 mL of deionized water. The solutions were stirred for 30 minutes, than allowed to sit for one hour. At this point, the pH of the solution was read using Thermo Electron Corporation Orion 550A® advanced pH/conductivity meter. Field samples were also sent to the British

Columbia Ministry of Forests and Range Research Branch laboratory and analyzed for total nitrogen (%N), total carbon (%C), and available phosphorus (P), cation-exchange capacity (CEC), and exchangeable aluminum (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and sodium (Na).

Statistical Analysis

The treatment factors of two *Vaccinium* species, four fungal inoculation options, and two soil amendments were applied in a complete factorial design. Individual plants in their microsites were used as the experimental units for all analyses. The effects of species, fungal inoculation, and soil amendment on the survival of the seedlings as well as the presence of mycorrhizal colonization were analyzed using three-way logistic ANOVA (LANOVA), using the GLM function (family=binomial) in the R statistical package (R Development Core Team 2007), examined at a significance level of 0.05. The effects of species, fungal inoculation, and soil amendment on the below-ground, above-ground, and total biomass were analyzed using three-way analysis of variance (ANOVA) examined at a significance level of 0.05. A square-root transformation was applied to the original data to ensure equal variance and normal distribution of the residuals. Differences between individual treatment combinations were analyzed using Tukey's post-hoc test with Bonferroni adjustments. All statistical analyses were performed with the R statistical computing program (R Development Core Team 2007).

3.2.2 Growth analysis

Berries from three more provenances of *V. membranaceum* and three more provenances of *V. myrtilloides* were collected during the summer of 2008 (Appendix 1). The berries were stored at 4°C until late September 2008, when the seeds were extracted by the

methods described in chapter 2. Seeds were air dried for four days, at which point they were surface sown on a 50:50 peat.sand growing medium and kept in greenhouse conditions with a 21°/10°C day/night temperature regime and natural light conditions. Growing trays were covered with clear plastic domes, placed on capillary matting, and watered every 7-10 days as necessary. Thirty days after sowing, at which point all provenances had seedlings emerging, the growing trays were inoculated with either an *R. ericae* solution or a sterile 20% PDA control solution. The *R. ericae* inoculum was prepared in the same manner as described above, with original cultures being diluted 3:1 with 20% PDA instead of 2:1. Again, the number of hyphal clusters per mL of inoculant solution was measured using a hemacytometer.

At days 34, 40, 48, 55, 62, 69, 76, 114, and 125 after sowing, 10 seedlings were selected from each treatment and provenance. The seedlings were dried for 24 hours at 70°C and then weighed. Due to the small size of the seedlings at this point, all ten seedlings were weighed together and an average dry mass per seedling was calculated. To provide increased replication, all provenances within a species were grouped together for analysis. Absolute growth rates and relative growth rates were calculated for the seedlings at each harvest interval using the equations (Y2-Yi)/(t2-ti) and **(lnY2**-lnYi)/(t2-ti), respectively, where Y2=dry weight in mg at the harvest date of interest (t2 in days since sowing) and Yi=dry weight in mg at the preceeding harvest day (ti in days since sowing) as described by Hunt (2003). After the final sampling date, five seedlings were selected from each treatment and provenance for staining and examination of mycorrhizal colonization, as described above.

Measurements of plant height and basal stem diameter, as well as counts of the number of leaves/plant were performed on 30 seedlings from each species x inoculation

treatment combination, for a total of 60 seedlings. Heights were measured to the nearest mm using a ruler and basal stem diameters were measured to the nearest 0.01 mm using digital calipers. Using the measured height and basal stem diameter, stem volume was calculated using the formula for the volume of a cylinder, V=l/37ir h, where V is stem volume, r is basal stem radius, and h is stem height.

Statistical analysis

A fungal inoculation treatment (inoculated or not) was applied to two *Vaccinium* species in a complete factorial design. The effect of seedling species, fungal inoculation, and number of days since sowing on the dry biomass of seedlings was also analyzed using a mixed-effects analysis of covariance (ANCOVA) model (R lme function) with seedling species and fungal inoculation, fitted as discrete variables, and days since sowing as a continuous variable, fitted as fixed effects and individual observation points fitted as random effects. This method was used in order to incorporate any autocorrelation, due to repeated measures, in the data into the results. A logarithmic transformation was used on the response variable of biomass to ensure equal variance and normal distribution of the residuals. All data was again examined at a significance level of 0.05. A post-hoc power analysis was performed on the data at each harvest date to determine the probability of type II errors occurring. Differences in absolute and relative growth rate were also examined using three-way ANCOVA using seedling species, fungal inoculation, and days since sowing as model inputs.

Individual observations of height, basal stem diameter, and number of leaves/plant for each plant were analyzed using two-way ANOVA to discern differences between seedling species and fungal inoculation treatment. A square-root transformation was used on the data

to ensure equal variance and normal distribution of the residuals. Differences between individual seedling species and fungal inoculation combinations were analyzed using Tukey's post-hoc test with Bonferroni adjustments. Power analyses were performed to determine the probability of type II errors.

3.3 Results

3.3.1 Field planting trials

Samples of the inoculum suspensions for each fungal species and the control inoculum were found to have significantly different levels of fungal clusters/mL when examined using a hemacytometer ($F_{3,20}$=4904.5, p=2.20 x 10^{-16}, R^2=0.9984) (Table 10). The inoculum suspensions of *R. ericae* and *O. maius* showed distinct hyphal clusters when viewed using light microscopy; however, the suspension of *Meliniomyces* sp. showed only smaller clusters of unidentifiable fungal matter. The numbers reported for *Meliniomyces* sp. indicate the number of these segments/mL instead of the number of hyphal clusters/mL. The control solution of sterile PDA showed no fungal matter when viewed under light microscopy.

Table 10. Mean number of fungal clusters per mL for three fungal suspensions and a sterile PDA control suspension. Numbers followed by the same lower-case letter indicate no significant differences found between groups.

Fungal suspension	Mean number fungal clusters/mL	Standard error
Rhizoscyphus ericae	90 000 a	5 774
Oidiodendron maius	40 000 b	5 164
Meliniomyces sp.	2 333 333 c	255 495
control (sterile PDA)	Od	0

Seedlings planted into field locations B and C (Table 9) experienced 100% mortality after 110 days in the field. The following analyses and results were conducted only using the numbers collected from field location A. Significant differences in survival were observed between seedlings of different species (p=2.55 x 10^{-7}); therefore further analyses were carried

out looking at the *V. membranaceum* and *V. myrtilloides* seedlings separately. *Vaccinium membranaceum* seedlings treated with different fungal inoculation treatments did not have any significant differences in survival percentages (p=0.431), nor did seedlings in amended vs. unamended plots (p=0.154). Significant differences in survival were observed between the different seed provenances (p=1.04 x 10^{-5}) (Table 11). Overall, *V. membranaceum* seedlings had survival rates of 6.0 + 2.15%.

Table 11. Percentage of *V. membranaceum* seedlings alive 110 days after being planted outdoors grouped by provenance, soil amendment, and fungal inoculant used.

Provenance	**Soil amendment**	**Fungal inoculant**	**Seedling survival (%)**
McBride Peak	LFH	*Rhizoscyphus ericae*	0.0
		Oidiodendron maius	0.0
		Meliniomyces sp.	0.0
		control	0.0
	none	*Rhizoscyphus ericae*	0.0
		Oidiodendron maius	0.0
		Meliniomyces sp.	0.0
		control	0.0
Mackenzie	LFH	*Rhizoscyphus ericae*	10.0
		Oidiodendron maius	0.0
		Meliniomyces sp.	0.0
		control	0.0
	none	*Rhizoscyphus ericae*	0.0
		Oidiodendron maius	10.0
		Meliniomyces sp.	0.0
		control	0.0
Teapot Mountain	LFH	*Rhizoscyphus ericae*	10.0
		Oidiodendron maius	10.0
		Meliniomyces sp.	40.0
		control	30.0
	none	*Rhizoscyphus ericae*	10.0
		Oidiodendron maius	0.0
		Meliniomyces sp.	20.0
		control	10.0

Vaccinium myrtilloides seedlings treated with different fungal inoculation treatments did not have any significant differences in survival percentages (p=0.240); however seedlings planted in amended plots had significantly higher survival percentages than those planted in

unamended plots (p=0.017). In this case, no significant differences in survival were observed among seedlings from different seed provenances (p=0.069) (Table 12). Overall, *V. myrtilloides* seedlings had survival rates of 22.1 ± 3.18%.

Table 12. Percentage of *V. myrtilloides* seedlings alive 110 days after being planted outdoors grouped by provenance, soil amendment, and fungal inoculant used.

Provenance	Soil amendment	Fungal inoculant	Seedling survival (%)
Aquatic Centre	LFH	*Rhizoscyphus ericae*	40.0
		Oidiodendron maius	40.0
		Meliniomyces sp.	50.0
		control	10.0
	none	*Rhizoscyphus ericae*	10.0
		Oidiodendron maius	30.0
		Meliniomyces sp.	20.0
		control	10.0
Coreless Park	LFH	*Rhizoscyphus ericae*	20.0
		Oidiodendron maius	40.0
		Meliniomyces sp.	30.0
		control	40.0
	none	*Rhizoscyphus ericae*	0.0
		Oidiodendron maius	40.0
		Meliniomyces sp.	20.0
		control	20.0
Mackenzie	LFH	*Rhizoscyphus ericae*	40.0
		Oidiodendron maius	10.0
		Meliniomyces sp.	20.0
		control	0.0
	none	*Rhizoscyphus ericae*	30.0
		Oidiodendron maius	0.0
		Meliniomyces sp.	10.0
		control	0.0

Vaccinium seedlings that survived being planted outdoors for 110 days showed no significant differences in total biomass between different species ($F_{5y}6_4$=0.247, £>=0.621) or among different fungal inoculation treatments ^5,64=2.012, *p*=0.123). Seedlings grown in amended plots, however, showed higher total biomass than seedlings growing in unamended plots *(F_5,64=8.592, p=0.005)* (Tables 13 &14, Figures 13, 14, & 15 a-b). Similar results were found for shoot biomass, with no differences seen between different species (F_{5i}64=0.951,

p=0.334) or inoculation treatments *(F5 64=0A36, p*=0.728), but with higher shoot biomass observed for seedlings growing in amended plots ($F_{5;}$(54=4.771, p=0.033) (Tables 13 & 14, Figures 13 & 14). Root biomass showed no differences between the two different species ($F_{5,6}$4=0.009, p=0.926); however significant differences were seen with amendment ***(F5164=*** 11.05, p=0.002) and fungal inoculation treatments ***(F5>64=***4.146, p=0.010). Again, seedlings in amended plots had higher root biomass than those in unamended plots. Seedlings inoculated with *R. ericae* and *O. maius* showed lower root biomass than control plants (r_5=-3.378,/?=0.001, f_5=-2.623,p=0.010 respectively) (Tables 13 & 14, Figures 13 & 14). Overall, *V. membranaceum* seedlings had a mean shoot biomass of 35.6 ± 9.2 mg, a mean root biomass of 42.3 + 9.9 mg, and a mean total biomass of 77.9 ±18.1 mg. *Vaccinium myrtilloides* seedlings had a mean shoot biomass of 44.3 ±6.1 mg, a mean root biomass of 42.2 ± 6.7 mg, and a mean total biomass of 86.5 ± 12.3 mg.

When examined for mycorrhizal colonization, only 4 out of 70 seedlings were found to be colonized. All of the colonized seedlings were growing in unamended plots *(p*=0.003); however, the colonization of the seedlings was not statistically influenced by species (p=0.758) or fungal inoculation treatment (p=0.190). In comparison, all root samples of *V. membranaceum* and V. *myrtilloides* plants grown from uninoculated rhizome cuttings in the greenhouse, grown for trials in chapter 2, were found to contain fungal colonization.

Soil pH for the outdoor plots ranged from levels of 6.09 to 8.11. Overall, amended plots had statistically significantly higher total N (Fi,$_4$=35.63,p=0.004), total C (F_M=42.58, p=0.003), available P (F_M=7.25, *p*=0.05), and exchangeable K (F_M= 13.43, p=0.021) and Mn (F,,4=61.47, p=0.001) than unamended plots (Table 15).

Table 13. Mean shoot, root, and total dry biomass (+ standard error, where applicable) of *V. membranaceum* seedlings 110 days after being planted outdoors, grouped by soil amendment and fungal inoculation treatment. Numbers in the same soil amendment group within a column followed by the same lower-case letter are not significantly different at the p=0.05 level.

Soil amendment	Fungal inoculant	Mean shoot biomass (mg)	Mean root biomass (mg)	Mean total biomass (mg)	n
LFH	*R. ericae*	10.4+1.1 b	18.7 +5.2 b	29.1 ± 6.3 b	2
	0. maius	6.5 b	3.6 b	10.1 b	1
	Meliniomyces	18.4 +4.3 b	29.1 + 7.8 b	47.5 ± 11.9 b	4
	control	74.8+ 13.8 a	108.0 + 5.6 a	182.8 ± 10.5 a	3
none	*R. ericae*	5.4 a	6.0 a	11.4 a	1
	O. maius	27.4 a	25.6 a	53.0 a	1
	Meliniomyces	81.7+ 36.6 a	51.7 ±22.9 a	133.4 ±59.5 a	2
	control	12.7 a	17.9 a	30.6 a	1

Table 14. Mean shoot, root, and total dry biomass (+ standard error) of *V. myrtilloides* seedlings 110 days after being planted outdoors, grouped by soil amendment and fungal inoculation treatment. None of the numbers in the same soil amendment group within a column were significantly different at the *p*=0.05 level.

Soil amendment	Fungal inoculant	Mean shoot biomass (mg)	Mean root biomass (mg)	Mean total biomass (mg)	n
LFH	*R. ericae*	59.1 ± 19.9	40.9 ± 14.8 a	100.0 ± 34.2 a	10
	O. maius	51.4 ± 12.5	37.8 ±7.7	89.2 ± 19.6	9
	Meliniomyces	52.7 ± 15.4	57.2 ± 17.2	109.9 + 32.2	11
	control	59.1 +32.0	104.4 + 44.2	163.5 ±73.9	5
none	*R. ericae*	14.5 ±3.6	9.5 ±5.2	24.0 ± 8.5	5
	O. maius	32.7 ± 6.9	25.3 ±8.4	58.0 ± 14.9	7
	Meliniomyces	27.7 + 7.7	26.9 ± 8.5	54.6 ± 15.4	5
	control	21.8 + 5.0	21.3 ±8.0	43.1 ± 13.0	3

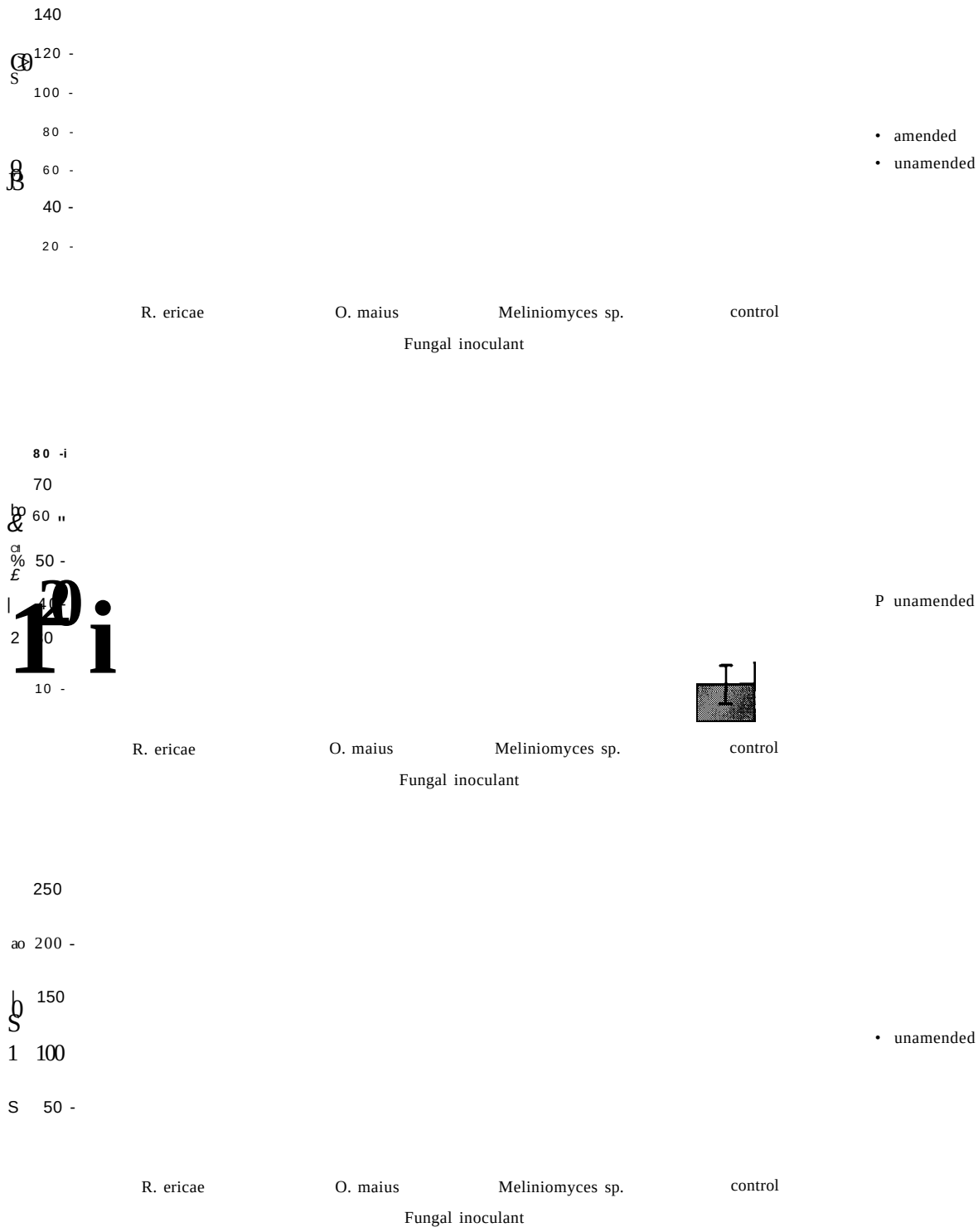

Figure 13. Mean dry shoot, root, and total biomass of *V. membranaceum* seedlings from amended and unamended plots, shown by fungal inoculant. Error bars represent ± one standard error.

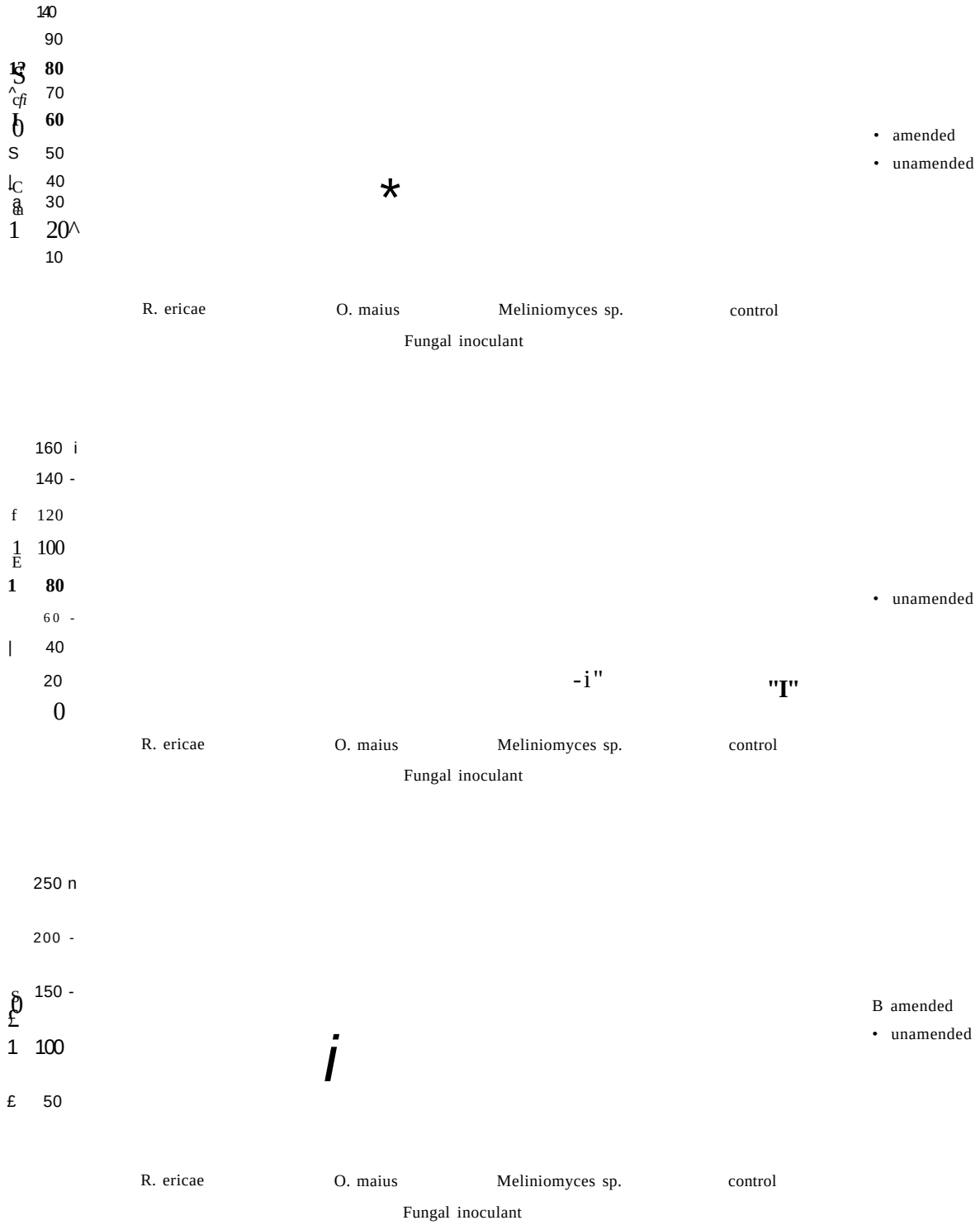

Figure 14. Mean dry shoot, root, and total biomass of *V. myrtilloides* seedlings from amended and unamended plots, shown by fungal inoculant. Error bars represent + one standard error.

Table 15. Chemical properties of amended and unamended soils at three *Vaccinium* planting sites.

Site	Amendment	pH (H_20)	Total C (%)	Total N (%)	Avail. P (ppm)
A	LFH	6.54	4.59	0.115	7.2
	none	7.26	0.66	0.033	4.1
B	LFH	6.09	6.65	0.135	9.7
	none	6.43	1.15	0.033	4.8
C	LFH	6.27	11.20	0.257	22.1
	none	8.11	0.54	0.021	5.0

Exchangeable cations ($cmol_c/kg$)

Site	Amendment	CEC	Al	Ca	Fe	K	Mg	Mn	Na
A	LFH	13.07	0.005	10.56	<0.001	0.291	1.94	0.035	0.235
	none	10.18	**0.001**	8.07	<0.001	0.229	1.58	0.002	0.291
B	LFH	18.24	0.012	14.70	<0.001	0.316	3.03	0.084	0.097
	none	13.15	0.008	10.27	0.002	0.230	2.55	0.003	0.082
C	LFH	29.42	0.013	23.82	0.002	0.366	4.95	0.140	0.133
	none	12.68	0.003	8.97	<0.001	0.170	3.45	0.003	0.085

In the supplemental planting of uninoculated seedlings, none of the 24 *V. membranaceum* seedlings had survived 60 days after being planted outdoors. In contrast, *V. myrtilloides* seedlings had an overall survival rate of 62.7%. Survival of these 64 seedlings was not significantly influenced by the soil amendment treatments (p=0.226) (Table 16). None of the surviving seedlings were found to have mycorrhizal colonization when examined using light microscopy.

Figure 15. Soil amendment treatments and washed seedlings, a) *V. membranaceum* seedling in unamended plot (75 days since outplanting). b) *V. myrtilloides* seedling in amended plot (31 days since outplanting). c) Washed *V. membranaceum* seedlings showing root systems, d) Washed *V. myrtilloides* seedlings showing root systems.

Table 16. Seedling survival, mean shoot, root, and total dry biomass (± standard error) of *V. myrtilloides* seedlings 60 days after being planted outdoors grouped by soil amendment. Numbers within a column followed by the same lower-case letter are not significantly different at the p=Q.05 level.

Soil amendment	Seedling survival *(%)*	Mean shoot biomass (mg)	Mean root biomass (mg)	Mean total biomass (mg)	n
LFH	67.3	58.5+ 5.5 a	39.5 ±4.5 a	98.0 ±9.4 a	35
none	55.8	54.9+ 4.6 a	25.5 ± 2.6 b	80.4 ±6.8 a	29

Looking at the surviving *V. myrtilloides* plants, seedlings growing in amended plots had significantly higher root biomass than those seedlings growing in unamended plots (Fi,52=6.02, p=0.0178). There were no significant differences in shoot biomass (F\^$_2$= 0.05,

/?=0.8317) or total biomass (/ri,52=l-44, p=0.2354) found between the soil amendment treatments (Figure 16). Overall, the second planting of *V. myrtilloides* seedlings had a mean shoot biomass of 57.1 ± 3.8 mg, a mean root biomass of 34.1 +3.1 mg, and a mean total biomass of 91.2 + 6.4 mg.

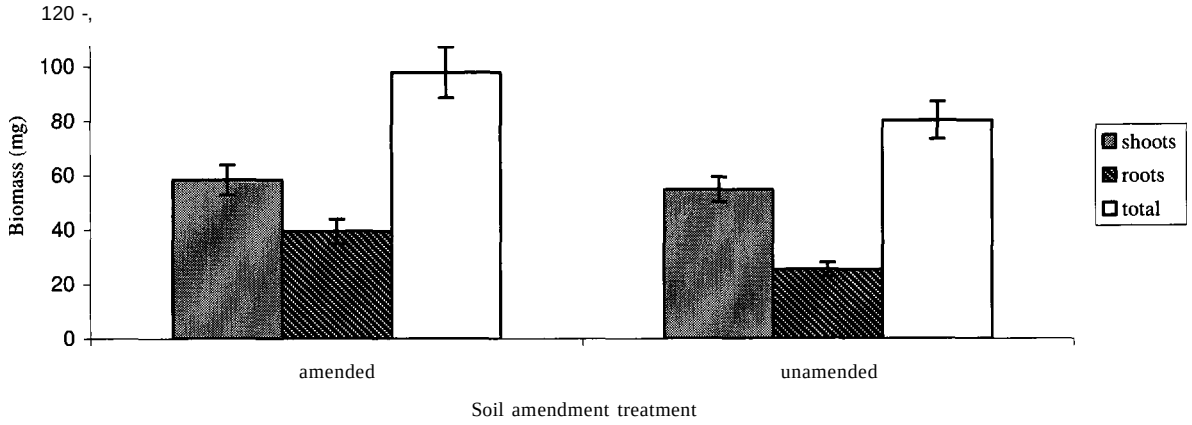

Figure 16. Mean dry shoot, root, and total biomass of *V. myrtilloides* seedlings from amended and unamended plots, 60 days after being planted outdoors. Error bars represent ± one standard error.

3.3.2 Growth analysis

Fungal inoculation treatment did not have any significant influence on the biomass of *Vaccinium* seedlings (Fi_{19}=0.173, p=0.689). Species differed in biomass (Fi_{19}=29.148, P=0.0006), with *V. membranaceum* seedlings having greater biomass measurements than *V. myrtilloides* seedlings, and older seedlings had higher biomass values than did younger seedlings (FI_{J95}=409.740, P=<0.0001) (Figures 17 & 18). Post-hoc power analyses indicated a 20.9% chance of detecting true differences among treatments at the p=0.05 level for day 34 after sowing, 42.1% for day 40 after sowing, 99.9% for day 48 after sowing, 34.1% for day 55 after sowing, 61.6% for day 62 after sowing, 32.2% for day 69 after sowing, 18.1% for day 76 after sowing, 94.7% for day 114 after sowing, and 88.9% for day 125 after sowing.

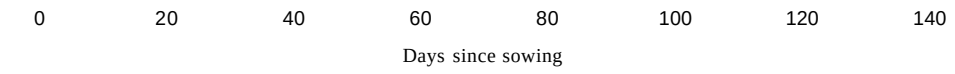

Figure 17. Mean dry biomass of 30 inoculated and 30 uninoculated greenhouse-grown *V. membranaceum* seedlings measured at nine intervals over 125 days. Error bars represent + one standard error.

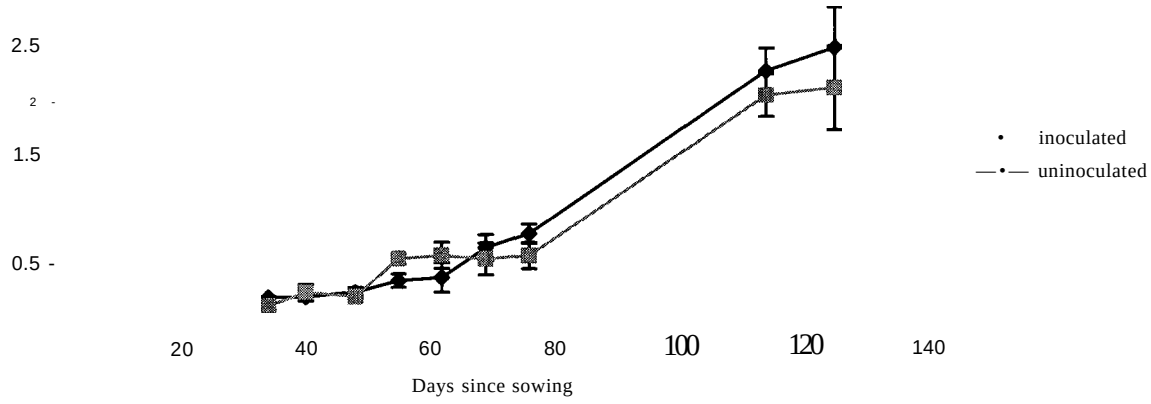

Figure 18. Mean dry biomass of 30 inoculated and 30 uninoculated greenhouse-grown *V. myrtilloides* seedlings measured at nine intervals over 125 days. Error bars represent ± one standard error.

The absolute growth rates for the *Vaccinium* seedlings did not vary significantly between different seedling species ($F_{3,104}$=0.673, p=0.414), fungal inoculations ($F_{3,104}$= 0.051, p=0.822), or sampling periods ($F_{3,104}$=2.48, p=0.119) (Figures 19 & 20).

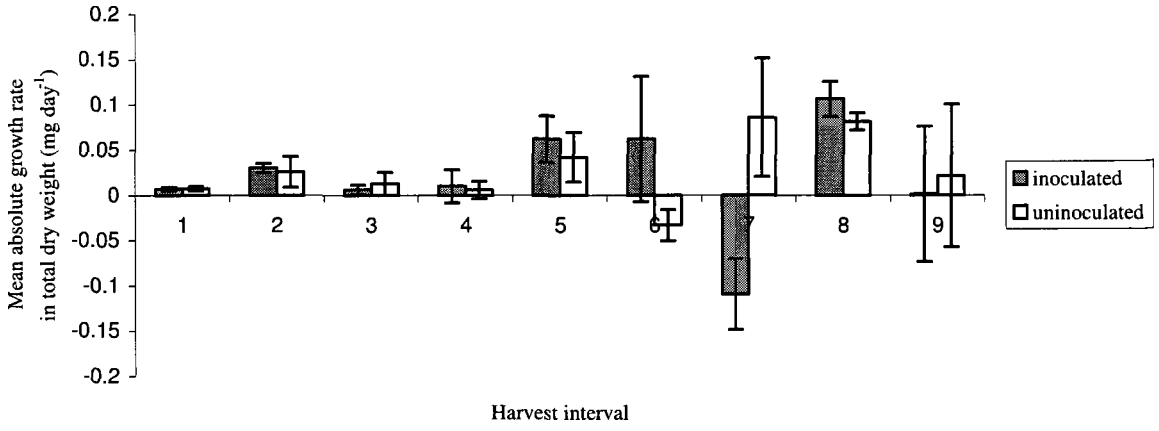

Figure 19. Mean absolute growth rate for inoculated and uninoculated *V. membranaceum* seedlings. For harvest intervals, see page 69 and x-axis in Figures 17 and 18. Error bars represent ± one standard error.

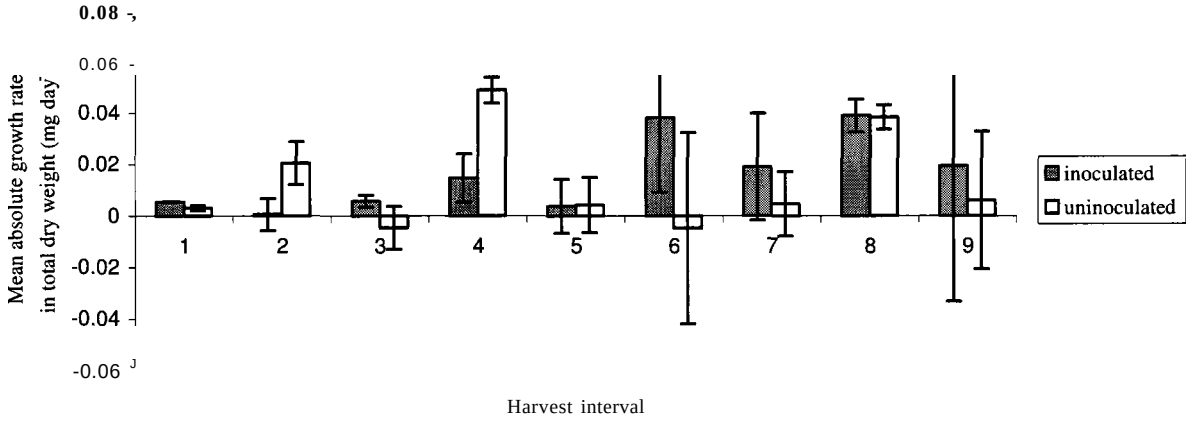

Figure 20. Mean absolute growth rate for inoculated and uninoculated *V. myrtilloides* seedlings. For harvest intervals, see page 69 and x-axis in Figures 17 and 18. Error bars represent ± one standard error.

Relative growth rates (Figures 21 & 22) also showed no significant differences between seedling species ($F_{3I}I_04=0.209$, p=0.885), fungal inoculations ($F_{3i}io4=0.343$, /?=0.560), or sampling periods (F_3,IO4=3. 305 x 10^{-5}, p=0.995).

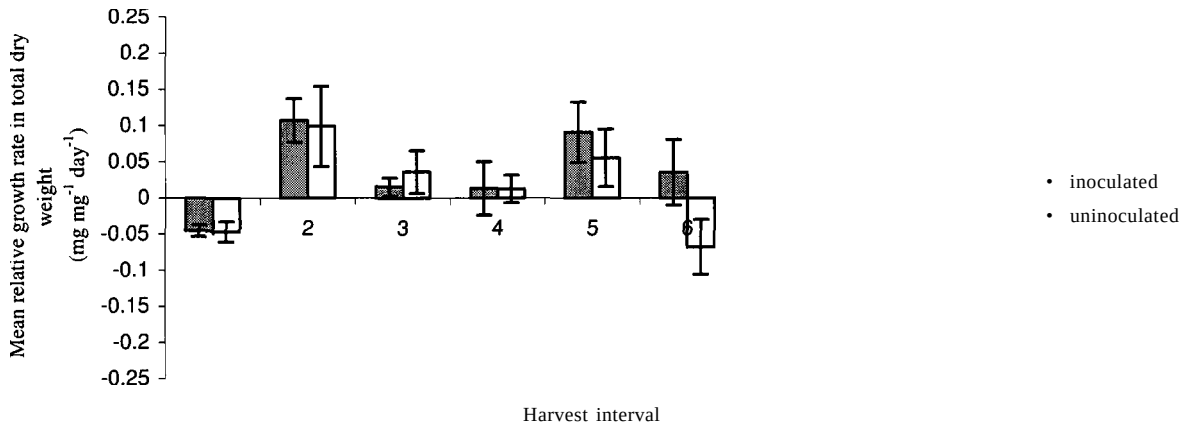

Figure 21. Mean relative growth rate for inoculated and uninoculated *V. membranaceum* seedlings. For harvest intervals, see page 69 and x-axis in Figures 17 and 18. Error bars represent + one standard error.

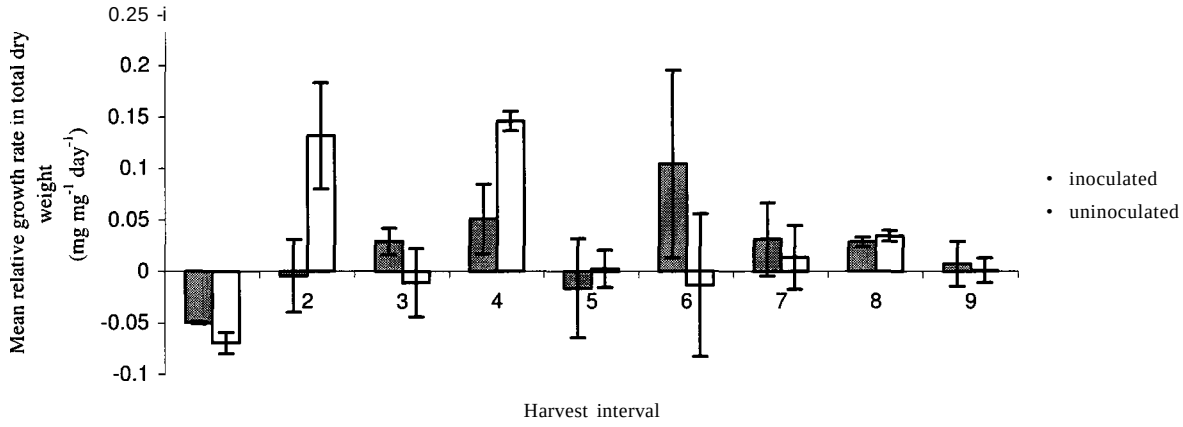

Figure 22. Mean relative growth rate for inoculated and uninoculated *V. myrtilloides* seedlings. For harvest intervals, see page 69 and x-axis in Figures 17 and 18. Error bars represent + one standard error.

The mean height of *Vaccinium* seedlings at 10 months was not significantly influenced by fungal inoculation (^/i 17=2.43, p=0.122); however species identity did influence the mean height (^2,117=39.64, p=5.628 x 10^{-9}), with *V. membranaceum* seedlings being taller than *V. myrtilloides* seedlings (Figure 23). Post-hoc power analysis gives a 99.9% chance of detecting true differences among treatments at the /?=0.05 level.

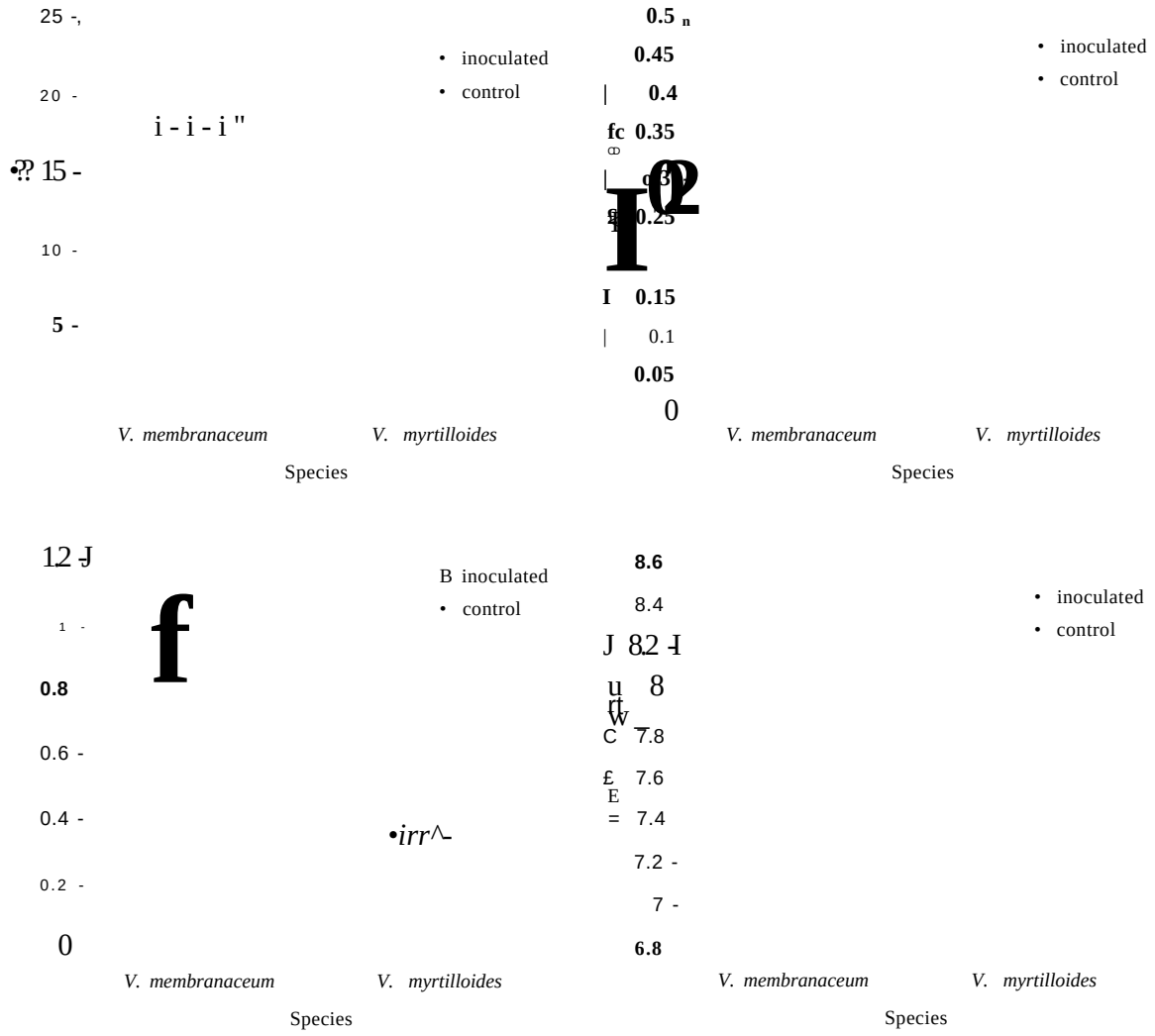

Figure 23. Mean height, basal stem diameter, stem volume, and number of leaves/plant of inoculated and control seedlings of *V. membranaceum* and *V. myrtilloides.* Error bars represent + one standard error.

The mean basal stem diameter of 10-month old seedlings also did not vary significantly between inoculated and control plants ($F_{2,m}=2.14$, $p=0.101$). *Vaccinium membranaceum* seedlings had significantly thicker stems than *V. myrtilloides* seedlings ($\chi^2_{2,117}=56.314$, $p = 1.369 \times 10^{-11}$) (Figure 23). Post-hoc power analysis gives a 99.9% chance of detecting true differences at the $p=0.05$ level.

The calculated stem volume of the seedlings was not significantly influenced by fungal inoculation (F_2,n7=0.324, *p=0.5705),* but was influenced by species identity. *Vaccinium membranaceum* seedlings had larger stem volumes than *V. myrtilloides* seedlings ($F_{2;}$in=89.81, p=4.479 x 10^{-16}) (Figure 23). Post-hoc power analysis gives a 99.9% chance of detected true differences at the p=0.05 level.

The number of leaves/plant for 10-month old V. *membranaceum* and *V. myrtilloides* seedlings did not vary significantly between seedling species (F2,i 17=0.62, p=0.432) or fungal inoculation (F2,n7=0.12, p=0.734) (Figure 23). Post-hoc power analysis gives only a 9.3% chance of detecting true differences at the *p=0.05* level for this trial.

None of the seedlings examined, of either *Vaccinium* species, were found to contain mycorrhizal colonization.

3.4 Discussion

In both outdoor trials, V. *myrtilloides* seedlings had higher survival levels than *V. membranaceum* seedlings. This was likely due to the size of the seedlings when they were planted outdoors. Although all the seedlings were the same age, *V. myrtilloides* seedlings ranged from 4-5 cm in height while V. *membranaceum* seedlings were only from 1.0-1.5 cm tall (Figure 15). Nielsen *et al.* (2004) found that *V. myrtilloides* occurred with higher frequency in clearcuts compared to *V. membranaceum.* This suggests that perhaps V. *myrtilloides* plants are better adapted to growing in more disturbed areas and can tolerate higher levels of direct sunlight, which would provide another explanation for the better performance of the *V. myrtilloides* seedlings in this study. *Vaccinium myrtilloides* is also more characteristic of the dry warm subzone of the Sub-Boreal Spruce biogeoclimatic zone

(SBSdw3), which is where the outdoor trials took place, whereas *V. membranaceum* is more typical of higher elevation and wetter biogeoclimatic units (Haeussler *et al.* 1990).

Fungal inoculation did not have any influence on the survival levels of either *Vaccinium* species. Past studies have reported varying results concerning the effectiveness of mycorrhizal fungal inoculation in *Vaccinium* species and other Ericaceae. Reich *et al.* (1982) reported no difference in growth and development of *Vaccinium* plants in Maryland that had been inoculated with mycorrhizal fungi and those that had not, whereas Powell (1982) in New Zealand, and Scagel (2005) in Oregon, reported increased shoot growth and dry matter production. In other studies from Maine and Pennsylvania, it was noted that the non-inoculated control plants had become colonized with mycorrhizal fungi from natural sources, making comparisons between the two groups difficult (Smagula and Litten 1989, Yang *et al.* 1998).

In our study, very few of the seedlings showed any mycorrhizal colonization, after 60 or 110 days, regardless of whether the fungi originated from the inoculation or from natural sources. Many studies report the successful mycorrhizal colonization of cultivated *Vaccinium* species following a variety of inoculation methods (Powell 1982; Reich *et al.* 1982; Litten *et al.* 1992; Goulart *et al.* 1995*b;* Yang *et al.* 1998; Yang *et al.* 2002; Scagel 2005; Kosola *et al.* 2007; Vohnik *et al.* 2007). The majority of these studies use micro-propagated plantlets as their test subjects. The two studies that indicated seedlings were used (Reich *et al.* 1982; Powell 1982) did not mention what age the seedlings were when the inoculation took place. The seedlings in this study were less than one year old, and the age or size of seedlings could play a vital role in the formation of ericoid mycorrhizal relationships. Young seedlings grow faster than adult plants and therefore one could expect

that they may use a higher proportion of the photosynthates they produce than adult plants would. Forming mycorrhizal relationships would decrease the amount of photosynthates available to the seedling for growth because the fungal partner would use some of them. If the seedlings are still at a point where conserving photosynthates is more important than increased access to soil nutrients, there may be a barrier to forming mycorrhizas. Small micro-propagated plants may not face this potential barrier because they grow in the adult growth form, which may no longer face these challenges. The physiological mechanisms of partner recognition and the initiation of ericoid mycorrhizal relationships are complex and not well understood (Smith and Read 2008). This hypothesis may explain why none of the younger seedlings used in the growth analysis showed any mycorrhizal colonization, while the rhizome cuttings all showed colonization.

Soil amendment had a positive influence on the survival levels and total biomass of the *Vaccinium* seedlings. These results correspond to work done by Yang *et al.* (2002) who found that pre-planting amendment with forest litter led to significantly higher dry weights for *V. corymbosum* plants than those plants with no amendment. Hamilton (2006) found natural *V. membranaceum* stands existing only on pre-burn forest floor plots, compared to pre-burn mineral soil plots. In this experiment, the soil plots amended with forest floor material had consistently lower pH levels and higher total N, total C, available P, effective CEC, and all exchangeable cations measured, with the exception of Fe (Table 15), compared to their non-amended counterparts. The greater availability of these nutrients favours higher unaided growth and survival.

The poor performance of the transplanted seedlings in open environments could, perhaps, have been expected. As noted above, all seedlings were exceptionally small, and

their root plugs of peat and sand were subject to rapid dehydration. The high pH levels of all of the soil plots would also not favour *Vaccinium* growth. The pH of soils growing natural field populations of *V. myrtilloides* ranges from 3.3 to 5.6 (Vander Kloet and Hall 1981). *Vaccinium membranaceum* field populations have been reported growing in soils of pH 3.0-6.0 (Haeussler *et al.* 1990). Ideal soil pH for growing cultivated *Vaccinium* species has been reported between the levels of 3.9 and 4.5 (Reich *et al.* 1982; Haynes and Swift 1985; Korcak 1986). The pH levels of the field sites in this study varied between 6.09 and 6.54 for amended plots, and from 6.43 to 8.11 for unamended plots, both well above the ideal levels for *Vaccinium* growth. High pH levels have also been shown to inhibit the formation of ericoid mycorrhizas (Haynes and Swift 1985). *Rhizoscyphus ericae* grown in media with a pH of 3.0 produce more mycelia than cultures grown in pH 7.0 media (Leake and Read 1990) and many ericoid mycorrhizal fungi have enzymes that are adapted to function at very low pH levels (Cairney and Meharg 2003). These findings suggest that ericoid mycorrhizal fungi are not viable at higher pH levels. A lack of viable fungal propagules explains the lack of mycorrhizal colonization and contributes to the poor survival of seedlings. The pH of the growing medium used in the greenhouse was 5.04, lower than the field sites but also above the ideal levels. If pH levels of the growing medium had been lower, the resulting seedlings may have been larger at the time of outplanting, which could have led to higher survival levels.

Another important variable affecting the survival of the seedlings outdoors was the weather. Seedlings were planted in mid-late June immediately before a spike in local daytime temperatures (Appendix 2). This increase in temperature caused water stress on the seedlings at a time when they were most vulnerable. Perhaps shading and mulching, as well

as organic amendment of the soil, would have helped reduce this moisture loss. Weather data for the 2008 growing season in Prince George are given in Appendix 2.

Both species of *Vaccinium* used in this study often recolonize sites disturbed by fire, primarily by means of rhizome sprouting (Moola and Mallik 1998, Hamilton 2006). Establishment on degraded or highly disturbed sites lacking a forest floor can be much slower and more difficult for *Vaccinium* (Haeussler *et al.* 2002). As such, these species are not effective "colonizers" in primary succession and fill an early role in secondary succession, primarily through their adaptations for post-fire recovery. These constraints may limit the viability of using *Vaccinium* species for reclaiming sites that have been subject to severe soil disturbance.

The provenance of the seedlings in the outdoor trials consistently influenced seedling survival. *Vaccinium* seeds were collected from varying locations and ecotypes throughout northern B.C. (Appendix 1). The seedlings that showed the poorest performance, notably all of the V. *caespitosum* and the V. *membranaceum* collected from McBride Peak, were grown from seed collected from the highest elevation locations. The *Vaccinium* plants in these higher elevation locations produced seed to grow plants adapted to the higher elevation conditions. When grown in the greenhouse and our field sites, these seedlings were no longer adapted to the growing conditions; consequently they did not have very high survival rates.

The growth curve data for *V. membranaceum* and *V. myrtilloides* show that neither of the species' growth was affected by fungal inoculation treatment. This is likely because the seedlings were not effectively colonized by mycorrhizal fungi, as described earlier. The unchanging rates of growth suggest that in the 125-day period after sowing, these species do

not reach a growth plateau. If the plants were monitored for a longer period of time, one would expect the growth rates to decrease when the plants reached maturity. The *V. membranaceum* seedlings sown in 2008 were observed to be larger than the *V. myrtilloides* seedlings sown in 2008. Although no measurements were taken, this is opposite to the seedlings sown in 2007, when the *V. myrtilloides* seedlings were larger than the *V. membranaceum* seedlings. The initial size of seedlings is partially due to parental vigour. Healthier parent plants are able to allocate more resources to building a healthy embryo and endosperm, giving the seedlings increased energy stores for initial growth (Banerjee *et al.* 2001). This component can be variable year-by-year, based on moisture and nutrient availability to the parent plants in any given growing season.

3.5 Conclusions and Recommendations

To the best of my knowledge, there are no existing published guidelines for the growth and transitioning of *V. membranaceum* or *V. myrtilloides* seedlings from indoor to outdoor growing conditions. Given the results of this study, I would recommend that anyone wanting to take *Vaccinium* seedlings from greenhouse conditions to outdoor areas should wait until the seedlings are at least two years old. This time period should allow seedlings to reach a size, both above- and below-ground, that would allow them to withstand outdoor conditions, while still maintaining the adaptability of young plants. Monitoring growth rates of seedlings over a longer period of time than was done in this study would allow a determination of the age when seedling growth rate begins to decrease. This time period would possibly indicate an age where seedlings are less vulnerable to external conditions and would be better able to survive outdoors. Seedlings should be planted early in the growing season, but after the risk of frost, possibly in mid-May for the Prince George area. Outdoor

planting areas composed mainly of mineral soil should be amended with local forest floor materials to improve soil aeration, water holding capacity, pH, CEC, and the level of nutrients available to the seedlings. Soils should be tested for pH prior to planting and, if necessary, additional amendments should be applied to reduce pH to levels of 3 to 4.

Mycorrhizal inoculation of *V. membranaceum* and *V. myrtilloides* seedlings under one year old does not appear to be effective in producing mycorrhizal colonization. Further research is required to determine the age at which these seedlings begin to form mycorrhizal associations and if the standard protocols used to grow fungi are adequate. Ideally, seedlings should be inoculated, with resulting colonization, before they are planted outside in order to take advantage of the benefits derived from ericoid mycorrhizas.

4.0 Synthesis and Application of Research

4.1 Synthesis of Research

In this study, seeds of *V. membranaceum, V. myrtilloides,* and *V. caespitosum,* and hardwood cuttings and rhizome cuttings of V. *membranaceum* and *V. myrtilloides* were tested under a variety of conditions. Seeds of these three species were found to vary in germination capacity based on species and collection location, but not with different seed storage times. Future studies on seed storage would do well to include more replicate samples to increase the power of statistical analysis as well as more storage types, including freezing. Adequate sample sizes should be determined through pre-testing power analyses. For example, using the measured variances, the minimum sample size needed to obtain an 80% power level in the seed storage treatment trials would have been 13. Further studies on seed germination could also include pre-sowing treatments, such as stratification, as the influences of these factors remain poorly understood for these three *Vaccinium* species.

Hardwood cuttings of *V. membranaceum* and *V. myrtilloides* rooted very poorly (<5%) and were not influenced by rooting medium or hormone formulation. Bottom heat appeared to be detrimental to above-ground vigour. Further studies using hardwood cuttings are advised to attempt to determine the optimal rooting hormone strength for these species, using a control group to see if the rooting hormones are having any effect at all, and to experiment with the timing of the collection of cuttings. Those propagating *V. membranaceum* or *V. myrtilloides* using hardwood cuttings should avoid using bottom heat, as it would appear to have adverse effects on the cuttings. In general, propagation from hardwood cuttings is not as effective as propagation from seed or rhizomes.

Sprouting of rhizome cuttings of *V. membranaceum* and *V. myrtilloides* was not influenced by the growing medium in which they were planted. The presence of bottom heat reduced the sprouting of the rhizome cuttings. Further studies of rhizome propagation could include increased types of growing media, as well as experimenting with the timing of rhizome collection and storage times. Those propagating either of these species using rhizome cuttings should avoid using bottom heat because of decreased sprouting.

To the best of my knowledge, there are no existing published guidelines for the growth and transitioning of *V. membranaceum* or *V. myrtilloides* seedlings from indoor to outdoor growing conditions. Given the results of this study, I would recommend that anyone wanting to take *Vaccinium* seedlings from greenhouse conditions to outdoor areas should wait until the seedlings are at least two years old. Monitoring growth rates of seedlings over a longer period of time than was done in this study would allow a determination of the age when seedling growth rate begins to decrease. This time period might indicate an age where seedlings are less vulnerable to external conditions and would be better able to survive outdoors. Seedlings should be planted early in the growing season, but after the risk of frost, possibly in mid-May for the Prince George area. Outdoor planting areas composed mainly of mineral soil should be amended with local forest floor materials to improve soil structure, water retention, and nutrient availability. Soils should be tested for pH prior to planting and, if necessary, additional amendments should be applied to reduce pH to levels of less than 5.

Mycorrhizal inoculation of *V. membranaceum* and *V. myrtilloides* seedlings under one year old does not appear to be effective in producing mycorrhizal colonization. Further research is required to determine the age at which these seedlings begin to form mycorrhizal associations and if the standard protocols used to grow fungi are adequate. Ideally, seedlings

should be inoculated, with resulting colonization, before they are planted outside in order to take advantage of the benefits derived from ericoid mycorrhizas.

4.2 Growing northern Vaccinium species in the greenhouse

4.2.1 Seedlings

Raising northern *Vaccinium* species from seed is one of the most cost-efficient methods of growing these plants in the greenhouse. Seed should be surface sown on a peat/sand medium with a pH level between 4.0 and 5.5. According to this research, V. *membranaceum* seeds can be expected to have a germination capacity of 5-66%, therefore over-sowing seeds at a minimum ratio of 2:1 would be advisable. *Vaccinium myrtilloides* seeds had a germination capacity of 13-29%, while *V. caespitosum* seeds had a germination capacity of 18-27%, and both species should be over-sown at a minimum ratio of 3:1. Seed trays should be kept with a 21°C/10°C day/night temperature regime and 12-15 hour of light/day. Covering the seed trays with clear plastic domes will decrease the amount of watering required. With adequate watering, and the occasional application of 20-20-20 N-P-K fertilizer, the seedlings should require very little maintenance until they are large enough to be transplanted into larger growing containers. *Vaccinium* seedlings grow slowly, and should ideally be left in the greenhouse for 2-3 years prior to being transplanted outdoors.

4.2.2 Rhizome cuttings

Rhizome cuttings are an especially attractive method of propagation where outplanting material is desired in a short period of time, and when plant salvage opportunities arise. Rhizome cuttings from northern *Vaccinium* species should be collected with care to avoid damaging the parent plant, unless conduced as a salvage operation prior to habitat destruction, as well as to ensure that the correct species of rhizome is being collected.

Rhizomes can be collected at any time during the fall and winter months, when the plant is dormant. When bringing rhizomes into the greenhouse it is important to either plant them right away or store them in an environment where they will not dry out, mold, or start to sprout on their own. Cold storage at 4°C is usually sufficient for meeting these requirements. Rhizome cuttings of at least 10 cm, or containing 2-3 nodes, should be planted into a peat/perlite medium or vermiculite. When using vermiculite care must be taken to ensure the cuttings do not dry out, as this will happen rapidly. If kept in a 21°C/10°C day/night temperature regime and 12-15 hour of light/day, the rhizome cuttings should sprout in 1-2 months. Rhizome cuttings of *V. membranaceum* and V. *myrtilloides* grown in these conditions should show a 70-80% sprouting rate. These cuttings grow more quickly than seedlings and would likely be ready to transplant outdoors after 6-12 months. Another alternative may be to plant unsprouted rhizome segments directly into new outdoor locations, although the efficacy of this method has not been tested.

4.2.3 Hardwood cuttings

The use of hardwood cuttings in propagating northern *Vaccinium* species should be avoided. The sheer numbers of cuttings that would have to be planted in order to result in a handful of viable plants make this method of propagation completely impractical at this time.

4.3 Transplanting northern Vaccinium to outdoor areas

When transplanting northern *Vaccinium* species from greenhouse conditions to outdoor plots, care must be taken to avoid placing too much stress on the plants. The plants should be gradually transitioned to outdoor conditions while still in their growing containers, either by being moved to a sheltered outdoor location or by being taken outdoors for increasing periods of time while still being taken in at night. If available, the application of

mycorrhizal inoculant at this time may benefit the plants, and certainly will not harm them. The chosen planting areas should be well prepared prior to the transplanting of the plants. Soil pH levels should be tested and amendments added, if necessary, to bring the pH levels to 4.0-5.0. The plants will also benefit from the addition of local forest floor material, preferably from an area where *Vaccinium* are growing naturally, to the planting area. *Vaccinium* plants should be planted after the risk of frost is over, but otherwise as early in the season as possible. After the transplanting, plants should be observed carefully for water stress, and watered as necessary. Depending on the size of the plants and the local weather, watering may be required daily. *Vaccinium* plants grown from seed will not start fruiting until they are 3-5 years old, those grown from rhizome cuttings may bear fruit at an earlier age. Growers must have patience when growing northern *Vaccinium* plants, but the end result will be worth the effort invested.

Northern *Vaccinium* species are not typically found as colonizers of harsh environments. Consequently, their use in reclamation and ecological restoration may be most suitable only where conditions of secondary succession, a more or less intact forest floor, prevail, such as in clearcuts and under power lines. Without soil amendments and careful monitoring for moisture stress, it can be quite difficult to establish these species in areas dominated by bare mineral soil or subsoil, such as decommissioned roads or landings, or pipeline excavations.

Literature Cited

Acree GE and Appleton BL. 2000. Mycorrhizal fungal inoculation of woody seed propagation substrate. Combined Proceedings International Plant Propagators' Society 50:551-555.

Alaback PB and Tappeiner JC, II. 1991. Response of western hemlock (*Tsuga heterophylla)* and early huckleberry (*Vaccinium ovalifolium*) seedlings to forest windthrow. Canadian Journal of Forest Research 21:534-539.

Albright M. 2004. Appendix C - Detailed propagation methods for beargrass, heather, huckleberry, and partridgefoot. WA: Olympic National Park.

Atlegrim O and Sjoberg K. 1996. Response of bilberry (*Vaccinium myrtillus*) to clear-cutting and single-tree selection harvests in uneven-aged boreal *Picea abies* forests. Forest Ecology and Management 86:39-50.

Banerjee SM, Creasy K, and Gertzen DD. 2001. Native Woody Plant Seed Collection Guide for British Columbia. British Columbia Ministry of Forests, Victoria BC. 147p.

Barclay-Estrup P. 1987. A new shrub for Ontario: Mountain Bilberry, *Vaccinium membranaceum,* in Pukaskwa National Park. Canadian Field-Naturalist 101(4):526-531.

Barney DL. 2003. Effects of light, surface sterilization, and fungicides on the germination of black huckleberry seeds. Small Fruits Review 2(2):73-80.

Barney DL. 1999a. Domestication of western huckleberries. Proceedings of the Northwest Center of Small Fruits Research 8:7-8.

Barney DL. 1999*b.* Growing western huckleberries. Moscow, ID: University of Idaho. 22p.

Barney DL. 1996. Seed and micropropagation of western huckleberries. Proceedings of the Northwest Center of Small Fruits Research 5:15-16.

Barney DL, Lopez OA, King E. 2007. Micropropagation of cascade huckleberry, mountain huckleberry, and oval-leaf bilberry using woody plant medium and Murashige and Skoog medium formulations. HortTechnology 17(3):279-284.

Barney DL, Shafii B, Price WJ. 2001. Cold stratification delays germination of black huckleberry seeds. HortScience 36(4):813.

Baskin CC, Milberg P, Andersson L, Baskin JM. 2000. Germination studies of three dwarf shrubs (*Vaccinium*, Ericaceae) of Northern Hemisphere coniferous forests. 78:1552-1560.

Berch SM, Allen TR, Berbee ML. 2002. Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. Plant and Soil 244:55-66.

Borland J and Bone M. 2007. Vegetative propagation of *Arctostaphylos* Adans.: Colorado style. Native Plants Journal 8(2):84-90.

Bradley R, Burt AJ, Read DJ. 1982. The biology of mycorrhiza in the Ericaceae VIII: The role of mycorrhizal infection in heavy metal resistance. New Phytologist 91(2): 197-209.

Bradley R, Burt AJ, Read DJ. 1981. Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris.* Nature 292:335-337.

Brundrett M, Kendrick B. 1990. The roots and mycorrhizas of herbaceous woodland plants I. Quantitative aspects of morphology. New Phytologist 114:457-468.

Cairney JWG and Meharg AA. 2003. Ericoid mycorrhiza: A partnership that exploits harsh edaphic conditions. European Journal of Soil Science 54:735-740.

Calmes MA and Zasada JC. 1982. Some reproductive traits of four shrub species in the black spruce forest type of Alaska. Canadian Field-Naturalist 96(l):35-40.

Chen HYH, Legare S, Bergeron Y. 2004. Variation of the understory composition and diversity along a gradient of productivity in *Populus tremuloides* stands of northern British Columbia, Canada. Canadian Journal of Botany 82:1314-1323.

Cole DN, Spildie DR. 2006. Restoration of Plant Cover in Subalpine Forests Disturbed by Camping: Success of Transplanting. Natural Areas Journal 26(2): 168-178.

Corkidi L, Evans M, Bohn J. 2008. An introduction to propagation of arbuscular mycorrhizal fungi in pot cultures for inoculation of native plant nursery stock. Native Plants 9(1):29-38.

Czesnik E and Eynard I. 1989. Mycorrhizal infection level in five cultivars of highbush blueberry (*Vaccinium corymbosum* L.). Agriculture, Ecosystems, and Environment 29:67-71.

Darrow GM and Scott DH. 1954. Longevity of blueberry seed in cool storage. Proceedings of the American Society for Horticultural Science 63:271.

Debnath SC. 2006. Influence of propagation method and indole-3-butyric acid on growth and development of in vitro- and ex vitro-derived lingonberry plants. Canadian Journal of Plant Science 86:235-243.

DesRochers A, Thomas BR, Buston R. 2004. Reclamation of roads and landings with balsam poplar cuttings. Forest Ecology and Management 199:39-50.

Dirr MA and Heuser CW. 1987. The reference manual of woody plant propagation. Athens, GA: Varsity Press, Inc. 239p.

Dobson A. 2000. A guide to propagation composts. Combined Proceedings International Plant Propagators' Society 50:148-152.

Erb WA, Draper AD, Swartz HJ. 1994. Combining ability for seedling root system size and shoot vigor in interspecific blueberry progenies. Journal of the American Society for Horticultural Science 119(4):793-797.

Erci§li S, E§itken A, Anapali O, §ahin U. 2005. Effect of substrate and IBA-concentration on adventitious root formation on hardwood cuttings of *Rosa dumalis.* Acta Horticulturae 690:149-152.

Eriksson O and Froberg H. 1996. "Windows of opportunity" for recruitment in long-lived clonal plants: Experimental studies of seedling establishment in *Vaccinium* shrubs. Canadian Journal of Botany 74:1369-1374.

Evans J, Wick D, Luna T. 2001. Propagation protocol for vegetative production of container *Vaccinium membranaceum* Dougl. Ex Hook, plants (800 mL containers); Glacier National Park, West Glacier, Montana. In: Native Plant Network. URL: http://www.nativeplantnetwork.org (accessed 21 September 2006). Moscow (ID): University of Idaho, College of Natural Resources, Forest Research Nursery.

Eynard I and Czesnik E. 1989. Incidence of mycorrhiza in four highbush blueberry cultivars in different soils. Acta Horticulturae 241:115-119.

Georgeson Botanical Garden. 2007. Georgeson Botanical Notes no. 34: How to germinate seeds of Alaska wild blueberries and lingonberries (lowbush cranberries). Fairbanks: University of Alaska Fairbanks. 2p.

Glass VM, Percival DC, Proctor JTA. 2005a. Tolerance of lowbush blueberries (*Vaccinium angustifolium* Ait.) to drought stress. I. Soil water and yield component analysis. Canadian Journal of Plant Science 85(4):911-917.

Glass VM, Percival DC, Proctor JTA. 2005*b*. Tolerance of lowbush blueberries (*Vaccinium angustifolium* Ait.) to drought stress. II. Leaf gas exchange, stem water potential and dry matter partitioning. Canadian Journal of Plant Science 85(4):919-927.

Glass VM, Percival DC, Proctor JTA. 2003. Influence of decreasing soil moisture on stem water potential, transpiration rate and carbon exchange rate of the lowbush blueberry *(Vaccinium angustifolium* Ait.) in a controlled environment. Journal of Horticultural Science and Biotechnology 78(3):359-364.

Gorecka K. 1979. The effect of growth regulators on rooting of Ericaceae plants. Acta Horticulturae 91:483-489.

Goulart BL, Demchak K, Yang WQ. 1997. Effect of cultural practices on field grown 'bluecrop' highbush blueberries, with emphasis on mycorrhizal infection levels. Acta Horticulturae 446:271-278.

Goulart BL, Demchak K, Yang WQ. 1995a. Organic matter and nitrogen level effects on mycorrhizal infection in 'bluecrop' highbush blueberry plants. Journal of Small Fruit Viticulture 3:151-164.

Goulart BL, Yang WQ, Demchak K. 1995Z?. Development of an inoculation system for studying mycorrhizal effects in highbush blueberry. Journal of Small Fruit Viticulture 3:193-201.

Haeussler S, Bedford L, Boateng JO, MacKinnon A. 1999. Plant community responses to mechanical site preparation in northern interior British Columbia. Canadian Journal of Forest Research 29(7): 1084-1100.

Haeussler S, Bedford L, Leduc A, Bergeron Y, Kranabetter JM. 2002. Silvicultural disturbance severity and plant communities of the southern Canadian boreal forest. Silva Fennica 36(l):307-327.

Haeussler S, Coates P, Mather J. 1990. Autoecology of common plants in British Columbia: A literature review. Victoria, B.C.: British Columbia Ministry of Forests and Forestry Canada. Report nr FRDA Report 158. 212-222 p.

Hamilton EH. 2006. Vegetation development and fire effects at the Walker Creek site: Comparison of forest floor and mineral soil plots. Victoria, B.C.: British Columbia Ministry of Forests and Range, Research Branch. Technical Report 026. 28p.

Haselwandter K. 1979. Mycorrhizal status of ericaceous plants in alpine and subalpine areas. New Phytologist 83:427-431.

Haynes RJ and Swift RS. 1985. Growth and nutrient uptake by highbush blueberry plants in a peat medium as influenced by pH, applied micronutrients and mycorrhizal inoculation. Scientia Horticulturae 27:285-294.

Hendershot WH, Lalande H, Duquette M. 2008. Chapter 16: Soil reaction and exchangeable acidity. In: Soil sampling and methods of analysis. Carter MR and Greynich EG, editors. 2nd ed. FL: CRC Press. 173-175 p.

Hill NM and Vander Kloet SP. 2005. Longevity of experimentally buried seed in *Vaccinium:* Relationship to climate, reproductive factors and natural seed banks. Journal of Ecology 93(6): 1167-1176.

Hunt R. 2003. Growth analysis, individual plants. In: Encyclopaedia of applied plant sciences. Murphy TB and Murray D, editors. London: Academic Press. 579-588 p.

Jacquemart A-L. 1996. Biological flora of the British Isles: *Vaccinium uliginosum* L. Journal of Ecology 84:771-785.

Jeliazkova E and Percival D. 2003. Effect of drought on ericoid mycorrhizae in wild blueberry (*Vaccinium angustifolium* Ait.). Canadian Journal of Plant Science 83:583-586.

Kender WJ. 1969. The effect of temperature and growth regulators on the propagation of the lowbush blueberry by rhizome cuttings. The Plant Propagator 15(2):3-5.

Kender WJ. 1967a. Rhizome development in the lowbush blueberry as influenced by temperature and photoperiod. Proceedings for the American Society of Horticultural Science 90:144-148.

Kender WJ. 19676. On the domestication of the lowbush blueberry. Fruit Varieties and Horticultural Digest 21:74-76.

Kender WJ. 1965. Some factors affecting the propagation of lowbush blueberries by softwood cuttings. Proceedings of the American Society of Horticultural Science 86:301-306.

Kerns BK, Alexander SJ, Bailey JD. 2004. Huckleberry abundance, stand conditions, and use in western Oregon: Evaluating the role of forest management. Economic Botany 58(4):668-678.

Korcak RF. 1986. Adaptability of blueberry species to various soil types: I. Growth and initial fruiting. Journal of the American Society of Horticultural Science 111(6):816-821.

Kosola KR, Workmaster BA, Spada PA. 2007. Inoculation of cranberry (*Vaccinium macrocarpon*) with the ericoid mycorrhizal fungus *Rhizoscyphus ericae* increases nitrate influx. New Phytologist 176:184-196.

Leake JR, Read DJ. 1990. Proteinase activity in mycorrhizal fungi I. The effect of extracellular pH on the production and activity of proteinase by ericoid endophytes from soils of contrasted pH. New Phytologist 115:243-250.

Leake JR, Shaw G, Read DJ. 1989. The role of ericoid mycorrhizas in the ecology of ericaceous plants. Agriculture, Ecosystems, and Environment 29:237-250.

Litten W, Smagula JM, Dalpe Y. 1992. Growth of micropropagated lowbush blueberry with defined fungi in irradiated peat mix. Canadian Journal of Botany 70:2202-2206.

Martino E, Turnau K, Girlanda M, Bonfante P, Perotto S. 2000. Ericoid mycorrhizal fungi from heavy metal polluted soils: Their identification and growth in the presence of zinc ions. Mycological Research 104(3):338-344.

Massicotte HB, Melville LH, Peterson RL. 2005. Structural characteristics of root-fungal interactions for five ericaceous species in eastern Canada. Canadian Journal of Botany 83:1057-1064.

McLean A. 1967. Germination of forest range species from southern British Columbia. Journal of Range Management 20:321-322.

Monni S, Salemaa M, Millar N. 2000a. The tolerance of *Empetrum nigrum* to copper and nickel. Environmental Pollution 109:221-229.

Monni S, Salemaa M, White C, Tuittila E, Huopalainen M. 20006. Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a Cu-Ni smelter, in southwest Finland. Environmental Pollution 109:211-219.

Moola FM and Mallik AU. 1998. Morphological plasticity and regeneration strategies of velvet leaf blueberry (*Vaccinium myrtilloides* Michx.) following canopy disturbance in boreal mixedwood forests. Forest Ecology and Management 111 :33-50.

Morrison S, Smagula JM, Litten W. 2000. Morphology, growth, and rhizome development of *Vaccinium angustifolium* Ait. seedlings, rooted softwood cuttings, and micropropagated plantlets. HortScience 35(4):738-741.

Nielsen SE, Munro RHM, Bainbridge EL, Stenhouse GB, Boyce MS. 2004. Grizzly bears and forestry II. Distribution of grizzly bear foods in clearcuts of west-central Alberta, Canada. Forest Ecology and Management 199(l):67-82.

Olsrud M, Michelsen A, Wallander H. 2007. Ergosterol content in ericaceous hair roots correlates with dark septate endophytes but not with ericoid mycorrhizal colonization. Soil Biology and Biochemistry 39:1218-1221.

Olsrud M, Melillo JM, Christensen TR, Michelsen A, Wallander H, Olsson PA. 2004. Response of ericoid mycorrhizal colonization and functioning to global change factors. New Phytologist 162:459-469.

Peterson RL, Massicotte HB, Melville LH. 2004. Mycorrhizas: Anatomy and cell biology. Ottawa: NRC Research Press. 173p.

Powell CL. 1982. The effect of the ericoid mycorrhizal fungus *Pezizella ericae* (Read) on the growth and nutrition of seedlings of blueberry (*Vaccinium corymbosum* L.). Journal of the American Society for Horticultural Science 107(6): 1012-1015.

Powell CL and Bagyaraj DJ. 1984. Effect of mycorrhizal inoculation on the nursery production of blueberry cuttings - a note. New Zealand Journal of Agriculture 27:467-471.

Powell CL and Bates PM. 1981. Ericoid mycorrhizas stimulate fruit yield of blueberry. HortScience 16(5):655-656.

R Development Core Team. 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Read DJ. 1984. Interactions between ericaceous plants and their competitors with special respect to soil toxicity. Aspects of Applied Biology 5:195-209.

Reich LA, Korcak RF, Thompson AH. 1982. Effects of two mycorrhizal isolates on highbush blueberry at two soil pH levels. HortScience 17(4):642-644.

Rice AV and Currah RS. 2005. *Oidiodendron*: A survey of the named species and related anamorphs *of Myxotrichum.* Studies in Mycology 53:83-120.

Rose R, Chachulski CEC, Haase DL. 1998. Propagation of Pacific Northwest native plants. Corvallis: Oregon State University Press. 248p.

Scagel CF. 2005. Inoculation with ericoid mycorrhizal fungi alters fertilizer use of highbush blueberry cultivars. HortScience 40(3):786-794.

Scagel CF. 2004. Enhanced rooting of kinnikinnick cuttings using mycorrhizal fungi in rooting substrate. HortTechnology 14(2): 1-9.

Shafii B and Barney DL. 2001. Drying and cold storage affect germination of black huckleberry seeds. HortScience 36(1): 145-147.

Sharpies JM, Meharg AA, Chambers SM. 2000a. Symbiotic solution to arsenic contamination. Nature 404:951-952.

Sharpies JM, Meharg AA, Chambers SM, Cairney JWG. 2000b. Mechanism of arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae.* Plant Physiology 124:1327-1334.

Smagula JM and Litten W. 1989. Effect of ericoid mycorrhizae isolates on growth and development of lowbush blueberry tissue culture plantlets. Acta Horticulturae 241:110-114.

Small E and Catling PM. 2005. Poorly known economic plants of Canada 45. Eastern huckleberries (*Gaylussacia* spp.) and western huckleberries (*Vaccinium* spp.). Canadian Botanical Association Bulletin 38(2): 15-23.

Smith SE and Read DJ. 2008. Ericoid mycorrhizas. In: Mycorrhizal symbiosis. Third ed. London: Academic Press. 389-419 p.

Stevens CM, Goulart BL, Demchak K, Hancock JF, Dalpe Y, Yang WQ. 1997. The presence, isolation, and characterization of ericoid mycorrhizal symbionts in two native and two commercial *Vaccinium* populations in central Pennsylvania. Acta Horticulturae 446:411-420.

Stevens M and Darris DC. 2000. NRCS plant guide: Black huckleberry *Vaccinium membranaceum* Dougl. ex Torr., Corvallis: United States Department of Agriculture. 4p.

Taiz L and Zeiger E. 2002. Plant physiology. 3rd ed. Sunderland, Massachusetts: Sinauer Associates, Inc. 690p.

Traveset A, Bermejo T, Wilson M. 2001. Effect of manure composition on seedling emergence and growth of two common shrub species of southeast Alaska. Plant Ecology 155:29-34.

Treu R, Laursen GA, Stephenson SL, Landolt JC, Densmore R. 1996. Mycorrhizae from Denali National Park and Preserve, Alaska. Mycorrhiza 6:21-29.

Turnau K, Mitka J, Kedzierska A. 1992. Mycorrhizal status of herb-layer plants in a fertilized oak-pine forest. Plant and Soil 143:148-152.

Vander Kloet SP. 1988. The genus *Vaccinium* in North America. Pub 1828. Research Branch, Agriculture Canada, Canadian Government Publication Centre, Ottawa. 20lp.

Vander Kloet SP and Hall IV. 1981. The biological flora of Canada 2. *Vaccinium myrtilloides* Michx., Velvet-leaf blueberry. Canadian Field-Naturalist 95(3):321-345.

Vander Kloet SP and Hill NM. 2000. *Bacca quo vadis*: Regeneration niche differences among seven sympatric *Vaccinium* species on headlands of Newfoundland. Seed Science Research 10(l):89-97.

Vander Kloet SP and Hill NM. 1994. The paradox of berry production in temperate species of *Vaccinium.* Canadian Journal of Botany 72:52-58.

Vohnik M, Albrechtova J, Vosatka M. 2005. The inoculation with *Oidiodendron maius* and *Phialocephala fortinii* alters phosphorous and nitrogen uptake, foliar C:N ratio and root biomass distribution in *Rhododendron* c.v. Azurro. Symbiosis 40:87-96.

Vohnik M, Fendrych M, Albrechtova J, Vosatka M. 2007. Intracellular colonization of *Rhododendron* and *Vaccinium* roots by *Cenococcum geophilum, Geomyces pannorum,* and *Meliniomyces variabilis.* Folia Microbiologica 52(4):407-414.

Whittle CA, Duchesne LC, Needham T. 1997. The importance of buried seeds and vegetative propagation in the development of postfire plant communities. Environmental Reviews 15:79-87.

Xiao G and Berch SM. 1996. Diversity and abundance of ericoid mycorrhizal fungi of *Gaultheria shallon* on forest clearcuts. Canadian Journal of Botany 74:337-346.

Xiao G and Berch SM. 1995. The ability of known ericoid mycorrhizal fungi to form mycorrhizae with *Gaultheria shallon.* Mycologia 87(4):467-470.

Xiao G and Berch SM. 1992. Ericoid mycorrhizal fungi of *Gaultheria shallon.* Mycologia 84(3):470-471.

Yang S, Bishop JG, Webster MS. 2008. Colonization genetics of an animal-dispersed plant *(Vaccinium membranaceum)* at Mount St Helens, Washington. Molecular Ecology 17:731-740.

Yang WQ, Goulart BL, Demchak K. 1998. Mycorrhizal infection and plant growth of highbush blueberry in fumigated soil following soil amendment and inoculation with mycorrhizal fungi. HortScience 33(7): 1136-1137.

Yang WQ, Goulart BL, Demchak K, Li Y. 2002. Interactive effects of mycorrhizal inoculation and organic soil amendments on nitrogen acquisition and growth of highbush blueberry. Journal of the American Society for Horticultural Science 127(5):742-748.

Appendix 1.

Table A1-1. Seed collection locations and dates for all seed-based trials

Seed Type	Location	Latitude/Longitude	Elevation (m)	Date collected	Date sown
V. caespitosum	Otway Trail, Prince George, B.C.	53°55'13.64" N 122°53'43.08" W	686	17/08/2005	31/10/2006
V. caespitosum	McBride Peak, near McBride, B.C.	53°20'14.45" N 120°07'05.89" W	2030	16/09/2006	31/10/2006
V. membranaceum	Pine Pass, Hwy 97 North, B.C.	55°20'48.59" N 122°37'14.37" W	998	25/08/2005	31/10/2006
V. membranaceum	Houston, B.C.	54°23'55.76" N 126°38'37.75" W	600	11/08/2006	31/10/2006
V. myrtilloides	Birch Creek Nursery, Prince George, B.C.	53°50'02.59" N 122°57'12.96" W	667	22/08/2005	31/10/2006
V. myrtilloides	Coreless Park, Prince George, B.C.	53°55'41.60" N 112°48'14.43" W	611	29/09/2006	31/10/2006
V. caespitosum	McBride Peak, near McBride, B.C.	53°20'14.45" N 120°07'05.89" W	2030	27/09/2007	22/11/2007
V. *caespitosum*	Morfee Mtn, near Mackenzie, B.C.	55°25'14.66" N 123°02'46.21" W	1560	19/08/2007	22/11/2007
V. membranaceum	Teapot Mtn, near Bear Lake, B.C.	54°19'35.88" N 122°40'45.84" W	896	25/08/2007	22/11/2007
V. membranaceum	Mackenzie, B.C.	55°09'06.48" N 123°12'28.19" W	733	19/08/2007	22/11/2007
V. membranaceum	McBride Peak, near McBride, B.C.	53°20'14.61" N 120°07'27.50" W	1937	27/09/2007	22/11/2007
V. myrtilloides	Coreless Park, Prince George, B.C.	53°55'41.60" N 112°48'14.43" W	611	23/08/2007	22/11/2007
V. myrtilloides	Aquatic Centre, Prince George, B.C.	53°54'25.37" N 112°48'29.57" W	607	23/08/2007	22/11/2007
V. myrtilloides	Mackenzie, B.C.	55°12'02.64" N 123°08'42.32" W	673	19/08/2007	22/11/2007
V. membranaceum	Smithers, B.C.	54°46'54.69" N 127°10'01.79" W	682	26/07/2008	27/09/2008
V. membranaceum	University of Northern British Columbia, Prince George, B.C.	53°53'12.02" N 122°48'55.24" W	713	05/08/2008	27/09/2008
V. membranaceum	McBride Peak, near McBride, B.C.	53°20'14.61" N 120°07'27.50" W	1937	13/09/2008	27/09/2008
V. myrtilloides	Coreless Park, Prince George, B.C.	53°55'41.60" N 112°48'14.43" W	611	03/08/2008	27/09/2008
V. myrtilloides	Kennedy Siding, near Bear Lake, B.C.	55°16'58.78" N 122°50'23.00" W	744	19/08/2008	27/09/2008
V. *myrtilloides*	Crooked River Provincial Park, near Bear Lake, B.C.	54°28'29.94" N 122°40'46.93" W	719	19/08/2008	27/09/2008

Appendix 2. Weather data for Prince George, **B**.C. from June-Sept. 2008.

All measurements are courtesy of Environment Canada Weather Office and were measured at Prince George International Airport (YXS), approximately 8 km southeast of the study site.

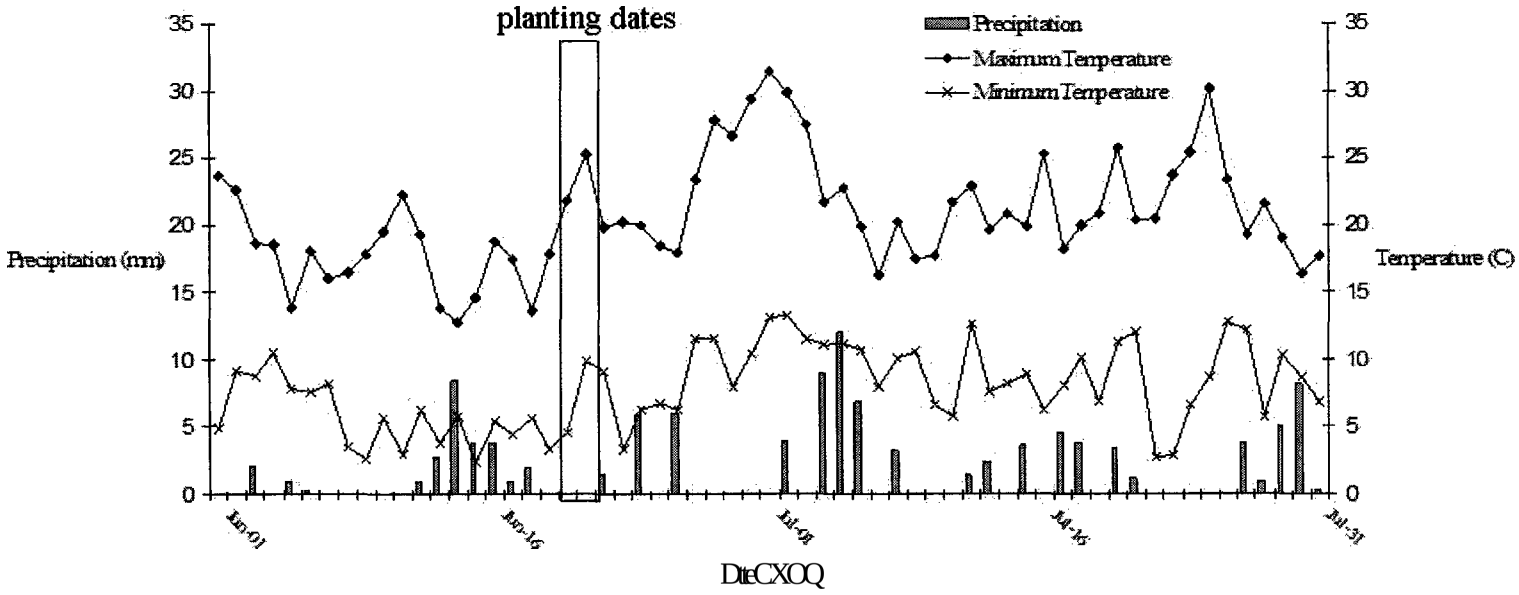

Figure A2-1. Precipitation, maximum and minimum daily temperatures for June 1, 2008-July 31, 2008

Appendix 2 con't

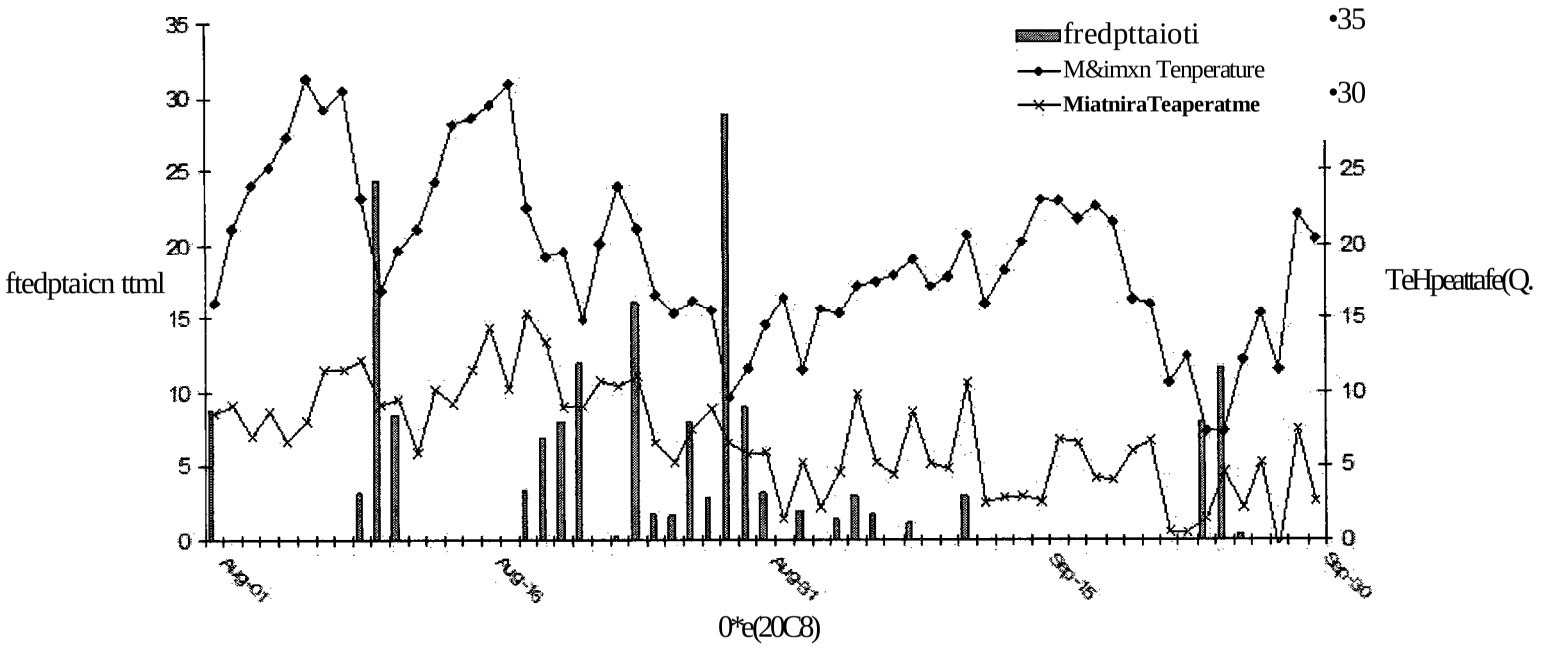

Figure A2-2. Precipitation, maximum and minimum daily temperatures for Aug. 1, 2008- Sept. 30, 2008.

Appendix 3. ANOVA tables

Abbreviations used:

df=degrees of freedom
SS=sum of squares
MS=mean of squares
AIC=Akaike's information criterion
LRT=likelihood ratio test
num=numerator
den=denominator

Chapter 2 Analyses:

Table A3-1. Total seed germination capacity (%) 2006 ANOVA table

Source	df	SS	MS	*F*	*P*
species	2	0.318	0.159	1.49	0.240
storage time	2	0.105	0.053	0.49	0.615
species x storage time	4	0.127	0.032	0.30	0.879
error	36	3.853	0.107		

Table A3-2. Number of seeds/berry ANOVA table[1]

Source	df	SS	MS	*F*	*P*
species	2	10.331	5.166	10.55	0.00001
provenance	5	6.716	1.343	2.74	0.025
error	72	35.249	0.490		

Table A3-3. Total seed germination capacity (%) 2007 ANOVA table[1]

Source	df	SS	MS	*F*	*P*
species	2	0.246	0.123	18.79	0.00001
provenance	3	0.103	0.034	5.24	0.006
error	24	0.157	0.007		

Table A3-4. Days to 50% of germination capacity 2007 ANOVA table[1]

Source	df	SS	MS	*F*	*P*
species	2	514.2	257.1	1.07	0.358
provenance	3	1146.1	382.0	1.60	0.217
error	24	5748.0	239.5		

Table A3-5. Total seed germination capacity (%) 2008 ANOVA table[1]

Source	df	SS	MS	*F*	*P*
species	1	0.732	0.732	75.47	0.000001
provenance	4	0.305	0.076	7.87	0.002
error	12	0.116	0.010		

Table A3-6. Days to 50% of germination capacity 2008 ANOVA table[1]

Source	df	SS	MS	F	P
species	1	68.06	68.06	0.27	0.610
provenance	4	838.89	209.72	0.85	0.523
error	12	2975.33	247.94		

Table A3-7. Hardwood cutting presence of roots (yes/no) LANOVA table[11]

Source	df	Deviance	AIC	LRT	P
species	1	46.940	56.942	0.002	0.964
medium	1	50.025	60.025	3.085	0.079
heat	1	47.742	57.742	0.802	0.370
hormone	2	52.597	60.597	5.657	0.059

Table A3-8. Hardwood cutting above-ground vigour (alive/dead) LANOVA table[11]

Source	df	Deviance	AIC	LRT	P
species	1	225.872	235.872	1.890	0.169
medium	1	224.821	234.821	0.838	0.360
heat	1	230.488	240.488	6.506	0.011
hormone	2	224.619	232.619	0.637	0.727

Table A3-9. April 15, 2008 rhizome cutting presence of sprouting (yes/no) LANOVA table[11]

Source	df	Deviance	AIC	LRT	P
medium	1	20.016	26.016	0.000	1.000
heat	1	53.841	57.841	33.825	6.03×10^{-9}

Table A3-10. July 15, 2008 rhizome cutting presence of sprouting (yes/no) LANOVA table[11]

Source	df	Deviance	AIC	LRT	P
medium	1	13.003	15.003	2.995	0.084

Table A3-11. October 11, 2008 rhizome cutting presence of sprouting (yes/no) LANOVA table"

Source	df	Deviance	AIC	LRT	P
species	1	42.510	46.510	1.315	0.252
medium	1	41.343	45.343	0.148	0.700

Table A3-12. Mean height comparison (mm) ANOVA table

Source	df	SS	MS	F	P
plant type	3	267.214	89.071	111.66	$<2.0 \times 10^{-16}$
species	1	0.974	0.974	1.22	0.271
plant type x species	3	44.998	14.999	18.80	1.765×10^{-10}
error	158	126.042	0.798		

removing non-significant terms gives:

Source	df	SS	MS	F	P
plant type	3	267.214	89.071	111.66	$<2.2 \times 10^{-5}$
plant type x species	14	45.972	11.493	14.41	4.741×10^{-10}
error	158	126.042	0.798		

Table A3-13. Mean basal stem diameter (mm) comparison ANOVA table

Source	df	SS	MS	F	P
plant type	3	5.695	1.898	327.01	<2.2 x 10^{1}"
species	1	0.172	0.172	29.695	1.909 x 10^{-7}
plant type x species	3	0.542	0.181	31.125	7.266 x 10^{16}
error	158	0.917	0.006		

Table A3-14. Mean # leaves/plant comparison ANOVA table

Source	df	SS	MS	F	P
plant type	3	65.354	21.785	158.37	<2.2 x 10^{16}
species	1	1.572	1.572	11.43	0.0009
plant type x species	3	10.135	3.378	24.56	4.248 x 10^{-13}
error	158	21.734	0.138		

Table A3-15. Mean stem volume (mm^3) comparison ANOVA table

Source	df	SS	MS	F	P
plant type	3	273.023	91.008	300.36	<2.2 x 10^{16}
species	**1**	13.161	13.161	43.44	6.207 x 10^{10}
plant type x species	3	21.215	7.072	23.34	4.470 x 10^{-12}
error	158	47.873	0.303		

Chapter 3 Analyses:

Table A3-16. Mean # fungal clusters/mL of inoculant ANOVA table

Source	df	SS	MS	F	P
fungi	3	724.73	241.58	4904.5	<2.2 x 10^{-16}
error	20	0.99	0.05		

Table A3-17. *V. membranaceum* seedling survival (alive/dead) 110 days after planting LANOVA table"

Source	df	Deviance	AIC	LRT	P
provenance	2	107.932	117.932	22.953	1.04 x lO'"
fungi	3	87.731	95.731	2.752	0.431
amendment	**1**	87.011	99.011	2.032	0.154

Table A3-18. *V. myrtilloides* seedling survival (alive/dead) 110 days after planting LANOVA table[11]

Source	df	Deviance	AIC	LRT	P
provenance	2	243.787	253.787	5.348	0.069
fungi	3	242.651	250.651	4.212	0.239
amendment	**1**	244.159	256.159	5.720	0.017

Table A3-19. Seedling shoot biomass (mg) after 110 days ANOVA table

Source	df	SS	MS	*F*	*P*
amendment	1	0.033	0.033	***4.11***	0.033
species	1	0.007	0.007	0.95	0.334
fungi	3	0.009	0.003	0.44	0.728
amendment x species	1	0.018	0.018	2.67	0.108
amendment x fungi	3	0.029	0.010	1.40	0.252
species x fungi	3	0.030	0.010	1.44	0.242
amendment x species x fungi	3	0.030	0.010	1.46	0.237
error	54	0.374	0.007		

removing non-significant terms gives:

Source	df	SS	MS	*F*	*P*
amendment	1	0.033	0.033	4.52	0.037
error	68	0.497	0.007		

Table A3-20. Seedling root biomass (mg) after 110 days ANOVA table

Source	df	SS	MS	*F*	*P*
amendment	1	0.076	0.076	11.05	0.002
species	1	0.00006	0.00006	0.01	0.926
fungi	3	0.086	0.029	4.15	0.010
amendment x species	1	0.007	0.007	0.97	0.328
amendment x fungi	3	0.048	0.016	2.32	0.085
species x fungi	3	0.011	0.004	0.51	0.677
amendment x species x fungi	3	0.011	0.004	0.55	0.650
error	54	0.373	0.007		

removing non-significant terms gives:

Source	df	SS	MS	*F*	*P*
amendment	1	0.076	0.076	10.99	0.002
fungi	3	0.084	0.028	4.04	0.011
error	65	0.452	0.007		

Table A3-21. Seedling total biomass (mg) after 110 days ANOVA table

Source	df	SS	MS	*F*	*P*
amendment	1	0.109	0.109	8.59	0.005
species	1	0.003	0.003	0.25	0.621
fungi	3	0.076	0.025	2.01	0.123
amendment x species	1	0.024	0.024	1.91	0.172
amendment x fungi	3	0.078	0.026	2.05	0.117
species x fungi	3	0.036	0.012	0.94	0.428
amendment x species x fungi	3	0.036	0.012	0.96	0.419
error	54	0.683	0.013		

removing non-significant terms gives:

Source	df	SS	MS	*F*	*P*
amendment	1	0.109	0.109	7.89	0.006
error	68	0.937	0.014		

Table A3-22. Soil pH ANOVA table

Source	df	SS	MS	F	P
amendment	1	0.029	0.029	3.91	0.119
error	4	0.030	0.007		

Table A3-23. Soil total C (%) ANOVA table

Source	df	SS	MS	F	P
amendment	1	7.541	7.541	42.58	0.003
error	4	0.708	0.177		

Table A3-24. Soil total N (%) ANOVA table

Source	df	SS	MS	F	P
amendment	1	4.441	4.441	35.63	0.004
error	4	0.499	0.125		

Table A3-25. Soil available P (ppm) ANOVA table

Source	df	SS	MS	F	P
amendment	1	1.263	1.263	7.25	0.055
error	4	0.697	0.174		

Table A3-26. Soil cation CEC ($cmol_c/kg$) ANOVA table

Source	df	SS	MS	F	P
amendment	1	0.335	0.335	3.62	0.130
error	4	0.371	0.093		

Table A3-27. Soil Al ($cmol_c/kg$) ANOVA table

Source	df	SS	MS	F	P
amendment	1	2.020	2.020	2.96	0.160
error	4	2.726	0.682		

Table A3-28. Soil Ca ($cmol_c/kg$) ANOVA table

Source	df	SS	MS	F	P
amendment	1	0.429	0.429	4.71	0.096
error	4	0.364	0.091		

Table A3-29. Soil Fe ($cmol_c/kg$) ANOVA table

Source	df	SS	MS	F	P
amendment	1	2.48×10^{-10}	2.428×10^{-10}	1.52×10	1.000
error	4	0.641	0.160		

Table A3-30. Soil K ($cmol_c/kg$) ANOVA table

Source	df	SS	MS	F	p
amendment	1	0.292	0.292	13.43	0.021
error	4	0.087	0.022		

Table A3-31. Soil Mg (cmolc/kg) ANOVA table

Source	df	SS	MS	F	P
amendment	1	0.091	0.091	0.49	0.524
error	4	0.749	0.187		

Table A3-32. Soil Mn ($cmol_c$/kg) ANOVA table

Source	df	SS	MS	*F*	*p*
amendment	1	16.792	16.792	61.47	0.001
error	4	1.093	0.273		

Table A3-33. Soil Na ($cmol_c$/kg) ANOVA table

Source	df	SS	MS	*F*	*p*
amendment	1	0.027	0.027	0.07	0.798
error	4	1.442	0.361		

Table A3-34. Supplemental seedling survival (dead/alive) 60 days after planting LANOVA table[11]

Source	df	Deviance	AIC	LRT	*p*
species	1	176.319	180.319	39.200	3.826 x10"[lu]
amendment	1	138.586	142.586	1.467	0.226

Table A3-35. Supplemental seedling shoot biomass (mg) after 60 days ANOVA table

Source	df	SS	MS	*F*	*p*
amendment	1	0.0001	0.0001	0.05	0.832
error	52	0.167	0.003		

Table A3-36. Supplemental seedling root biomass (mg) after 60 days ANOVA table

Source	df	SS	MS	*F*	*p*
amendment	1	0.016	0.016	6.02	0.018
error	52	0.134	0.003		

Table A3-37. Supplemental seedling total biomass (mg) after 60 days ANOVA table

Source	df	SS	MS	*F*	*p*
amendment	1	0.007	0.007	1.44	0.235
error	52	0.268	0.005		

Table A3-38. Growth analysis (mg) mixed-effects model ANCOVA table

Source	num df	den df	*F*	*P*
species	1	8	29.15	0.0006
inoculation	1	8	0.17	0.689
time	1	92	409.74	<0.0001
species x inoculation	1	8	0.01	0.944
species x time	1	92	0.18	0.673
inoculation x time	1	92	0.00	0.984
species x inoculation x time	1	92	0.02	0.877

removing non-significant terms gives:

Source	num df	den df	*F*	*P*
species	1	10	35.07	0.0001
time	1	95	423.86	< 0.0001

Table A3-39. Absolute growth rate (mg day"[1]) ANOVA table

Source	df	SS	MS	F	P
species	1	0.002	0.002	0.67	0.414
inoculation	1	0.0002	0.0002	0.05	0.822
time	1	0.008	0.008	2.48	0.119
species x inoculation	1	0.0009	0.0009	0.27	0.602
species x time	1	0.0008	0.0008	0.25	0.620
inoculation x time	1	6.007 x 10"[7]	6.007 x 10^{-7}	0.0002	0.989
species x inoculation x time	1	0.0010	0.0010	0.30	0.586
error	100	0.319	0.003		

Table A3-40. Relative grow h rate (mg mg"[1] day"[1]) ANOVA table

Source	df	SS	MS	F	P
species		0.0002	0.0002	0.21	0.885
inoculation		0.002	0.002	0.34	0.560
time		2.36 x 10"[7]	2.36 xlO"[7]	3.31 x 10	0.995
species x inoculation		0.0003	0.0003	0.05	0.832
species x time		0.0003	0.0003	0.04	0.838
inoculation x time		0.0010	0.0010	0.14	0.712
species x inoculation x time		0.002	0.002	0.32	0.571
error	100	0.714	0.007		

Table A3-41. Height of seedlings (mm) ANOVA table

Source	df	SS	MS	F	P
species	1	12.628	12.628	39.64	5.628 x 10"[9]
inoculation	1	0.774	0.774	2.43	0.122
species x inoculation	1	0.764	0.319	2.40	0.124
error	116	36.950			

removing non-significant terms gives:

Source	df	SS	MS	F	P
species	1	12.628	12.628	38.72	7.719 x 10"[9]
error	118	38.489	0.326		

Table A3-42. Basal stem diameter (mm) of seedlings ANOVA table

Source	df	SS	MS	F	P
species	1	0.173	0.173	56.31	1.369 x 10 "
inoculation	1	0.008	0.008	2.74	0.101
species x inoculation	1	0.00009	0.00009	0.03	0.867
error	116	0.357	0.003		

removing non-significant terms gives:

Source	df	SS	MS	F	P
species	1	0.173	0.173	55.95	1.444 x 10"[11]
error	118	0.365	0.003		

Table A3-43. Number of leaves/plant of seedlings ANOVA table

Source	df	SS	MS	F	P
species	1	0.056	0.056	0.62	0.432
inoculation	1	0.011	0.011	0.12	0.734
species x inoculation	1	0.001	0.0011	0.01	0.914
error	116	10.518	0.0907		

Table A3-44. Stem volume (mm^3) of seedlings ANOVA table

Source	df	SS	MS	*F*	*P*
species	1	2.756	2.756	89.37	4.527×10^{-10}
inoculation	1	0.010	0.010	0.32	0.571
species x inoculation	1	0.034	0.034	1.10	0.296
error	116	3.577	0.031		

removing non-significant terms gives:

Source	df	SS	MS	*F*	*P*
species	1	2.756	2.756	89.81	3.479×10^{-lft}
error	118	3.621	0.031		

[1] interactions not tested due to insufficient degrees of freedom

[n] interactions not testable on logistic models