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Growth and Restoration Potential of Five Nitrogen Fixing Species on Soil Amendments of Waste Rock and Materials from Victor Mine

A thesis submitted in partial fulfillment of the requirements for the Honour's degree of Bachelor of Science in Biology

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ABSTRACT

Understanding plant-soil interactions in mine waste soils is essential for effective, low maintenance remediation to occur. Nitrogen fixing plants are especially important for remediation of mine overburden because it contains little to no organic material or nitrogen. Inoculating plants with nitrogen fixing actinomycete bacteria and mycorrhizal spores is essential in mine soils. Using a growth chamber I examined the growth of four native shrub species: and one non-native legume species on a soil created by mine waste overburden by a 20:60:20 ratio of FPK:CPK:silt (fine processed kimberlite, coarse processed kimberlite), 40% peat (by volume) and a control soil of vermiculite and peat. They were inoculated with two types of materials and supplemented with phosphorus fertilizer. There were significant differences in plant dry mass between species and soil type and a significant interaction between species and soil type. For the species *Alnus rugosa* and *Trifolium repens*, the difference in the number of nodules, root, shoot, and total masses was large for control and kimberlite soil; As well *Alnus crispa* did not survive in any kimberlite soils. For *Alnus crispa*, *Alnus rugosa*, and *Trifolium repens* there was a large reduction in plant growth of the kimberlite soil. This was a likely consequence of the non-optimal high pH in kimberlite soil and small seed size for both *Alnus* sp and *T. repens*. However for the species *Shepherida canadensis* and *Elaeagnus commutata* the difference in root, shoot, and total masses, and number of nodules was much smaller for kimberlite and control soils, suggesting they are candidate species of nitrogen fixing shrubs for reclamation at Victor mine in HBL.

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INTRODUCTION

Managing primary succession becomes essential on disturbed and barren sites, such as mining wasteland, in order to establish a sustainable and beneficial vegetative cover (Luken 1990). Bioavailable nitrogen is often low at these sites and must be somehow added to promote successional processes. Nitrogen-fixing species of herbaceous plants and shrubs are essential as early successional species in land restoration projects because they provide long-term and sustainable sources of bio-available nitrogen (Luken 1990). Due to the low carbon: nitrogen in the litter of these species, decomposition is quite rapid allowing nutrients to more quickly become available for other plant species (Robb 2001). Legumes are often used as N-fixing herbs because of fast colonization, but shrubs with actinorhizal associations are also commonly used N-fixing species in restoration (Roy *et al* 2007; Khamzina *et al* 2009; Dommergues 1997). Actinorhizal shrubs not only provide N-fixation, but also perennial and complex structure to barren or disturbed lands to moderate the surface microclimates and provide habitat and food for wildlife (Roy *et al* 2007; Dommergues 1997).

Actinorhizal species are so called because they are capable of forming root associations with: actinomycete bacteria *Frankia* and a mycorrhizal association (Tjepkema & Torrey 1979). In the actinomycete association, *Frankia* sp. convert atmospheric nitrogen into the usable plant form ammonium, while in exchange the actinomycete receives photosynthates from the plant (Huss-Danell 1997; Schwintzer & Tjepkema 1990; Roy *et al* 2007). The roots are also colonized by a mycorrhizal component which has been shown to enhance nutrient and water uptake for the host and increases the success of a plant when being transplanted from laboratory to the field (Monzon & Azcon 2001; Robb 2001; Quoreshi 2008). This mycorrhizal component is also important for structural development of new soils (Gardner 1986). The three associations are

termed tripartite symbioses because of the three part symbioses (*Frankia*-Plant-Mycorrhizae) however mycorrhizal associations can occur by ectomycorrhizal fungi and/ or arbuscular mycorrhizae, potentially forming tetrapartite symbioses (Molina *et al* 1994).

In North America, actinorhizal shrubs include members of Betulaceae (*Alnus*), Coriariaceae (*Coriaria*), Datiscaceae (*Datisca*), Elaeagnaceae (*Elaeagnus* and *Shepherdia*), Myricaceae (*Myrica* and *Comptonia*), Rhamnaceae (*Adolphia* and *Ceanothus*), Rosaceae (*Cercocarpus, Dryas*, and *Purshia*) (Dommergues 1997; Cruz-Cisneros & Valdes 1990). Alders (*Alnus* sp.) have been well researched on their nitrogen fixing capacity. They are widespread and native in North America, and are quite tolerant to disturbance (Roy *et al* 2007, Hibbs & Cromack 1990; Quoreshi 2008; Tarrant & Trappe 1971; Molina *et al* 1994). They can fix approximately 40-300 kg N/ha/yr depending on the species, thus helping to develop nitrogen pools in soils otherwise lacking in available nitrogen (Roy *et al* 2007, Hibbs & Cromack 1990; Berry & Torrey 1985; Quoreshi 2008). Alder species have been shown to colonize shortly after disturbance (Seeds & Bishop 2009; Hibbs & Cromack 1990; Gardner 1986; Robb 2001). Species of *Elaeagnus commutata* and *Shepherdia canadensis* have been documented on disturbed slopes with low available nitrogen and low organic matter (Moore 1964). *Elaeagnus* sp. have been known to live up to one-hundred years, and are very tolerant to saline soils and harsh environments and therefore are commonly planted for slope stabilization in Russia, Asia, and selected places in Europe (Kiseleva & Chindyaeva 2011). For some actinorhizal species, they can adapt to habitats that are unlike their native habitat and are often tolerant to several pollutants (Dommergues 1997).

Mycorrhizal and *Frankia* sp. are sometimes shown to be species specific; there has been much recent work done on the taxonomy for *Frankia* and mycorrhizal species (Roy *et al* 2007; Huguet *et al* 2001). Generally *Frankia* have diverged into three major clusters, each of which

form a symbioses with a few specific families of actinorhizal plants (Huguet *et al* 2001). In terms of Northern Ontario species, *Frankia* sp. in the phylogenetic cluster 3 have been shown to successfully form a symbioses with the Elaeagnaceae family (*Shepherdia* sp. and *Elaeagnus* sp.), and *Alnus* sp. and Myricaceae are successful nodulated by *Frankia* sp. belonging to cluster 1. Interesting to note, *Frankia* sp. obtained from the nodules of *Myrica* sp. were sometimes capable of nodulating plant families specific to other phylogenetic clusters of *Frankia* such as Elaeagnaceae, possibly due to the primitive evolution of Myricaceae to create actinorhizal plants (Huguet *et al* 2001). Nodulation in *Alnus* sp. can occur in as little as 1-2 weeks after inoculation (Huss-Danell 1997), however depending on the laboratory conditions nodulation may not occur for several weeks after inoculation (Berry & Torrey 1985; Roy *et al* 2007).

Sites such as peatlands, mine wastes, and areas made barren after an extreme disturbance have been shown to have few nitrogen-fixing bacteria and mycorrhizae strains in the soil necessary for symbioses to occur (Seeds & Bishop 2009; Huss-Danell & Frey 1986). The inoculation of nitrogen fixing shrubs is extremely important to increase their success when transplanted into the field, and because distribution of *Frankia* spores is still not well known. It is believed *Frankia* are not wind dispersed, but rather by means of animals, humans, or water (Huss-Danell *et al* 1999). Phosphorus limitation and excessive nitrogen in a substrate can inhibit nodulation (Koo *et al* 1996). Also soil characteristics like pH and particle size (affecting pore space) will influence nodulation and successful establishment of these plants (Dommergues 1997; Molina *et al* 1994; Markham 2005).

Actinorhizal shrubs have the potential to aid in the development of sustainable plant communities in subarctic mines following mine closure. At the DeBeers Victor mine in the Hudson Bay Lowland, mining wastes include fine and coarse processed kimberlite (FPK & CPK), coarse limestone, silt overburden and peat. In 2010, there was over 350 ha of new upland

area disturbed by the mining process and requiring remediation (Bergeron, Laurentian Msc candidate, unpublished data). Soil restoration plans intend to utilize the waste rock and overburden on site to create a suitable substrate for plant growth. The soils produced from these mixes have similar characteristics to serpentine soils. They are alkaline (pH ~8.5) and have low total and bio-available P and also a Ca:Mg ratio less than 1, however they have do not have high concentrations of metals and are plentiful with K (Bergeron, Laurentian Msc candidate, unpublished data) unlike serpentine soils (Brady *et al* 2005). The successful establishment of nitrogen fixing shrubs will assist in soil structural development, nitrogen and organic inputs, and therefore a reduction and stabilization in pH (Lefrancois *et al* 2010) creating increased nutrient availability for later species (Luken 1990; Roy *et al* 2007). Rouble (2011) and Bergeron (Laurentian Msc candidate, unpublished) were able to create a functional soil amendment using the waste materials from Victor mine. The soil mix which produced the highest biomass, for *Poa pratensis* (Kentucky Blue grass) and *Trifolium repens* (White Clover) in two independent experiments was a 20:60:20 mix of FPK:CPK:silt respectively with an additional 40% peat by volume (Bergeron, Laurentian Msc candidate, unpublished data; Rouble 2011). Both these experiments received mild N, P, K fertilizations.

I will test the hypothesis that four nitrogen fixing shrubs and a legume can grow in a soil amendment of 20:60:20 of FPK:CPK:silt and 40% peat from Victor Mine. The four shrub species are *Elaeagnus commutata* (Silverberry), *Shepherdia canadensis* (Buffaloberry), *Alnus crispa* (Green Alder) and *Alnus rugosa* (Speckled Alder); they are widespread and native to the Hudson Bay Lowlands (Riley 2003). These species have been chosen due to their ability to form tripartite symbioses with *Frankia* and one or more mycorrhizal association (Gardner 1986; Koo *et al* 1996). The legume *Trifolium repens* (White Clover), which is not native to the region, but is used here as a useful comparison species, forms a symbiotic association with the diazotroph

Rhizobium. I will also test the hypothesis that inoculation with infusions made from litter, roots, and nodules around mature plants will increase nodulation compared to infusions made from only the litter from mature plants. I expect that using the 20:60:20 soil medium, the actinorhizal shrubs will be capable of growth but likely demonstrate some nutrient deficiencies. Due to the differences between plant families and species, there will likely be interactions between species, soil type, and/ or inoculants types.

MATERIALS AND METHODS

This growth chamber experiment was set up as a random block factorial design with two soil mixes, five nitrogen-fixing plant species, two inoculation methods, and five blocks. There were five replicates for a total of one hundred plants.

Mining wastes, FPK, CPK, silt and peat were collected from Victor mine and shipped to Sudbury in 2011. All materials were dried in a growth chamber at approximately 30°C for 3 weeks. The FPK and silt required crushing in order to create particle sizes true to their original analysis (approximately 3.9-62.5µm; Bergeron, Laurentian Msc candidate, unpublished data) and to create a more homogenized substrate for mixing. We prepared two soil mixes. The first was a 20:60:20 ratio of FPK:CPK:silt to which was added, 40% peat by volume. The second soil mix was a mixture of vermiculite and peat, respectively, in a 60:40 ratio by volume. Each soil mix was placed in a cement mixer and mixed for 30 minutes. The soils were placed in 4" pots with ~500 mL volume.

Five N-fixing species were grown*: Alnus rugosa, Alnus crispa, Trifolium repens, Elaeagnus commutata* and *Shepherdia canadensis*. *A. rugosa* and *A.crispa* seeds were collected from Sudbury at Lake Laurentian Conservation areas and Onaping at A.Y. Jackson trail and were allowed to air dry. *E. commutata* and *S. canadensis* were from obtained from Sheffield's Seed Co. (Locke, NY 13092 USA; www.sheffields.com). *T. repens* seeds were from obtained from Southview Greenhouse Growers in 2011. This legume species was used as a comparison with the native shrubs and native legumes. *E. commutata* and *S. canadensis* required a cold stratification for approximately 60-90 days so were steeped in warm water for 24 hours, placed in Petri dishes on moistened paper towelling, wrapped in aluminum foil, then placed in a fridge

at approximately 5°C in mid-August until mid-October (60 day stratification). *A. crispa* was also placed in a fridge at 5°C for 15 days before planting.

Two inoculation slurries were created from natural sources, following the procedures from Quoreshi (2008), with some variation. Inoculation method A was made from a slurry of nodules, leaf litter, and roots from mature plants while inoculation method B had only leaf litter added the slurry. To make the slurry, 6 L of leaf litter from under *T. repens, A. rugosa, A. crispa, S. canadensis,* and *Myrica gale,* were added to 42 L of distilled water. The solution was mixed, let rest for 24 hours and separated into two. 50mL of nodules from species: *T. repens, A. rugosa, M. gale,* and *A. crispa,* were crushed with a sterilized mortar and pestle and added to inoculation method A, along with 30mL of crushed roots from *S. canadensis* (no visible nodules present on root). The two inoculation slurries were prepared immediately after the collection of the litter and humus materials since desiccation of spores would kill the microorganisms (Quoreshi 2008). Before plant inoculation, the litter was sieved out by a 2 mm sieve.

A. rugosa, A. crispa, E. commutata, and *S. canadensis* were germinated in trays containing a 50:50 mixture of AllTreat Farms Premium Potting Mix® and vermiculite. Trays were placed in a growth chamber (BioChambers AC-60) with 16 hours of light per day under a mix of fluorescent and incandescent bulbs, with day and night temperatures of 25°C and 15°C, respectively. When a seedling showed 1-2 true leaves, it was carefully transplanted to a prepared pot. *T. repens* seed was sowed directly into soil treatments and thinned to one plant after germination. Inoculation occurred immediately after seedling transplant and for *T. repens* after true leaves were present. Plants were inoculated three times with 60 mL of inoculant. The plants were grown under the same conditions stated above. The trays and pots were watered daily with distilled water. The pots were also watered with 50mL of a 2.31g/L, 1% (KH_2PO_4)

fertilizer three times a week for six weeks. If a seedling died following transplantation, another seedling was transplanted to the pot, to a maximum of three transplantations.

The pH of the control and kimberlite soils were determined using Fisher Scientific-accumet, Water proof hand held meters: serial # 479962; mfg code # 01X262910FSAP85. The soil samples were mixed with two parts water to one part soil, stirred and left for approximately fifteen minutes and stirred again before measuring.

Data Collection and Analysis

After 6 weeks of plant growth, the plants were carefully removed from the pots and the amount of nodulation was quantified by counting visible nodules per plant. The length of the shoot and roots were measured, then separated and placed in separate paper bags and dried in a drying oven at 80°C for a minimum 48 hours. The total plant biomasses, root masses, and shoot masses were measured to 0.1mg accuracy and the root: shoot mass ratios calculated. The data was analyzed using a 2×2×5 factorial ANOVA with a random block design, using SPSS software and Tukey's post hoc analysis at a significance of $P<0.05$. Normality and was verified by residual plots and confirmed with KS tests, homogeneity of variance was confirmed by residual vs. predictive plots. Non-normal data was transformed using square root transformations.

RESULTS

For *A. crispa* survival was low. Out of twenty plants, only seven plants survived and none of the plants that survived were grown in kimberlite soils. The plants that did not survive were transplanted up to three times.

Total Mass

The data was square root transformed to meet assumptions of the ANOVA. The total biomass was significantly different for plant species and soil medium ($P<0.001$, Table 1), but there was no significant differences related to the soil inoculation methods ($P=0.171$). The mean total plant mass was greatest for *T. repens* (1.05g), followed by *E. commutata* (0.46g); the total mass for *A. rugosa* (0.11g), *A. crispa* (0.06g), and *S. canadensis* (0.10g) were not considered significantly different (Fig. 1). Plants on average also grew better in control than on kimberlite soils. However, there was also a significant interaction between soil type and species ($P<0.001$). *A. rugosa* and *T. repens* grew much better in the control soil as compared to the other species, whereas *S. canadensis* and *E. commutata* had smaller differences in total mass between soil types.

Table 1. Factorial ANOVA for square-root transformed total plant mass. Significant effects are in bold ($P<0.05$).

Source	df	MS	*F*	*P*
Block	4	0.005	0.3	0.897
Inoculant	1	0.001	0.06	0.810
SoilType	1	2.697	135.9	**<0.001**
Species	4	1.489	75.0	**<0.001**
SoilType * Species	3	0.828	41.7	**<0.001**
Inoculant * SoilType	1	0.007	0.3	0.565
Inoculant * Species	4	0.007	0.4	0.828
Inoculant * SoilType * Species	3	0.002	0.1	0.962
Error	63	0.020		

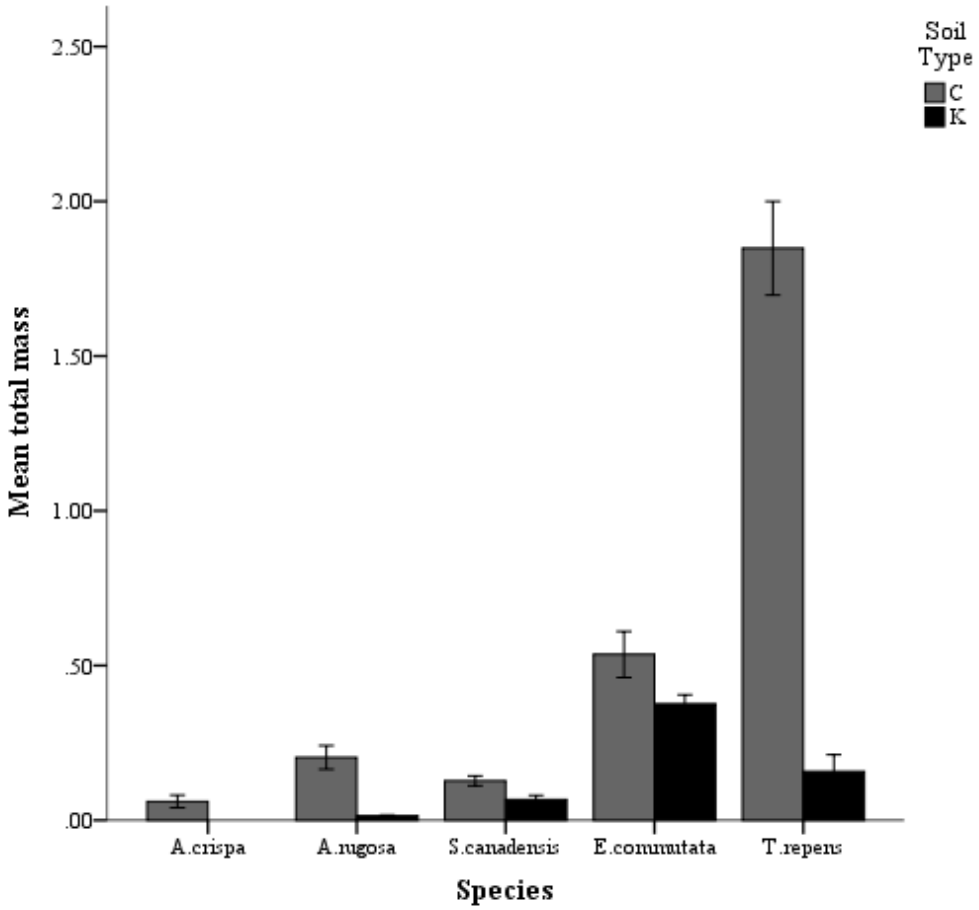

Figure 1. Effect of plant species and soil type (C-control; K-kimberlite) on the total plant mass (g) (mean ± SE). Raw data are displayed, but they were square-root transformed for analysis.

Root Mass

The dry root mass showed a similar story. Dry root mass was significantly different for plant species and soil medium (P<0.001, Table 2), with again no significant differences in root mass due to soil inoculation methods (P=0.760). The root mass was greatest for *T. repens* (0.36g), followed by *E. commutata* (0.16g); the root masses for *A. rugosa* (0.03g), *A.crispa* (0.02g), and *S. canadensis* (0.03g) were not considered significantly different (Fig. 2). The plants grown in the control soil had a significantly larger root mass in comparison to the kimberlite soil. There was a significant interaction between soil type and species (P<0.001); *A.*

rugosa and *T. repens* again had much greater root mass from the control soil than in the kimberlite soil while *S canadensi*s and *E commutata* had much less differences between the soils. All other interactions were non-significant (P>0.05).

Table 2. Factorial ANOVA for dry root mass on untransformed data. Significant effects are in bold (P <0.05).

Source	df	MS	*F*	*P*
Block	4	0.001	0.3	0.853
Inoculant	1	0.000	0.1	0.760
SoilType	1	0.509	151.7	**<0.001**
Species	4	0.370	110.2	**<0.001**
SoilType * Species	3	0.339	100.9	**<0.001**
Inoculant * SoilType	1	0.000	0.002	0.964
Inoculant * Species	4	0.001	0.3	0.899
Inoculant * SoilType * Species	3	0.001	0.2	0.921
Error	63	0.003		

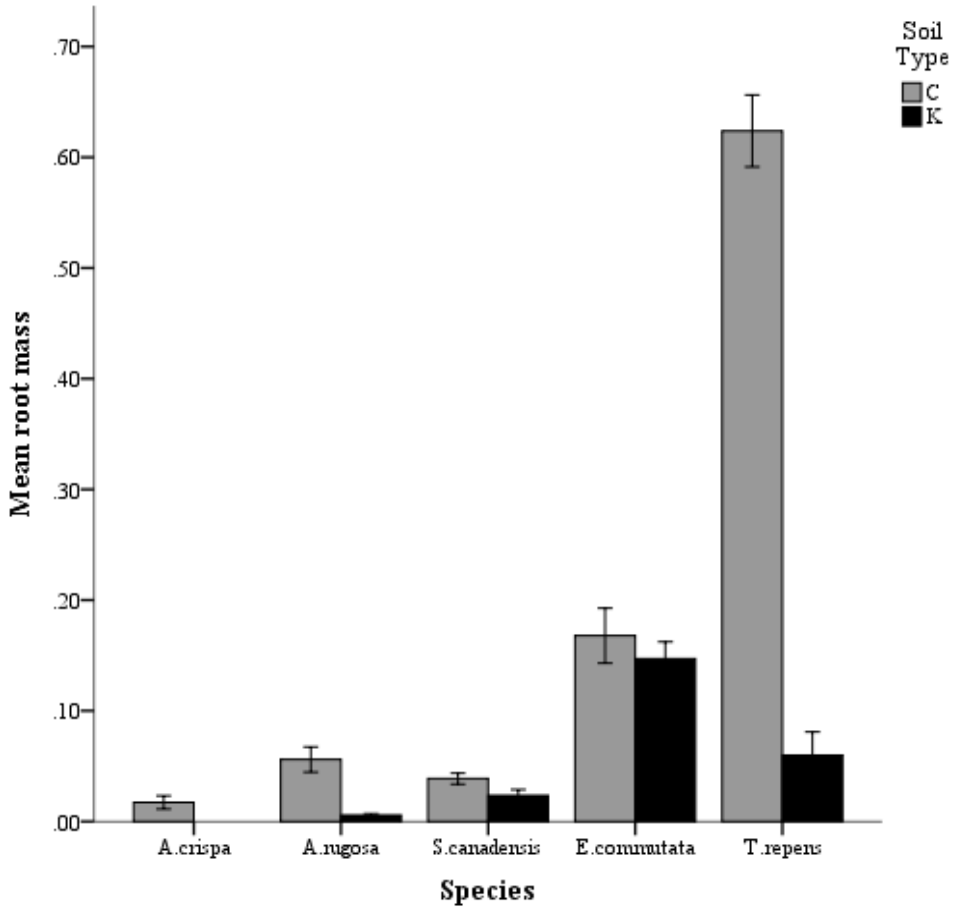

Figure 2. Effect of plant species and soil type (C-control; K-kimberlite) on root dry mass (g). Standard error bars represent +/- one standard error.

Shoot Mass

The data was square root transformed to meet the assumptions of the ANOVA. Shoot mass again showed the same pattern as for total and root biomass. The dry shoot mass was significantly different between plant species and soil medium ($P<0.001$, Table 3), but again there was no significant differences due to soil inoculation methods ($P=0.929$). The mean dry shoot mass was greatest for *T. repens* (0.69g), followed by *E. commutata* (0.30g); the shoot masses for *A. rugosa* (0.08g), *A. crispa* (0.04g), and *S. canadensis* (0.07g) were not considered significantly different (Fig. 3). There was another significant interaction between soil type and species

(*P*<0.001). *T. repens* had the largest difference in mean shoot mass from the control soil and kimberlite soil than the other species.

Table 3. Factorial ANOVA for square root transformed dry shoot mass. Significant effects are in bold (*P* <0.05).

Source	Df	MS	F	P
Block	4	0.002	0.3	0.889
Inoculant	1	4.23E-05	0.008	0.929
SoilType	1	0.638	119.1	**<0.001**
Species	4	0.326	60.9	**<0.001**
SoilType * Species	3	0.265	49.4	**<0.001**
Inoculant * SoilType	1	0.001	0.2	0.638
Inoculant * Species	4	0.001	0.1	0.980
Inoculant * SoilType * Species	3	0.000	0.04	0.988
Error	63	0.005		

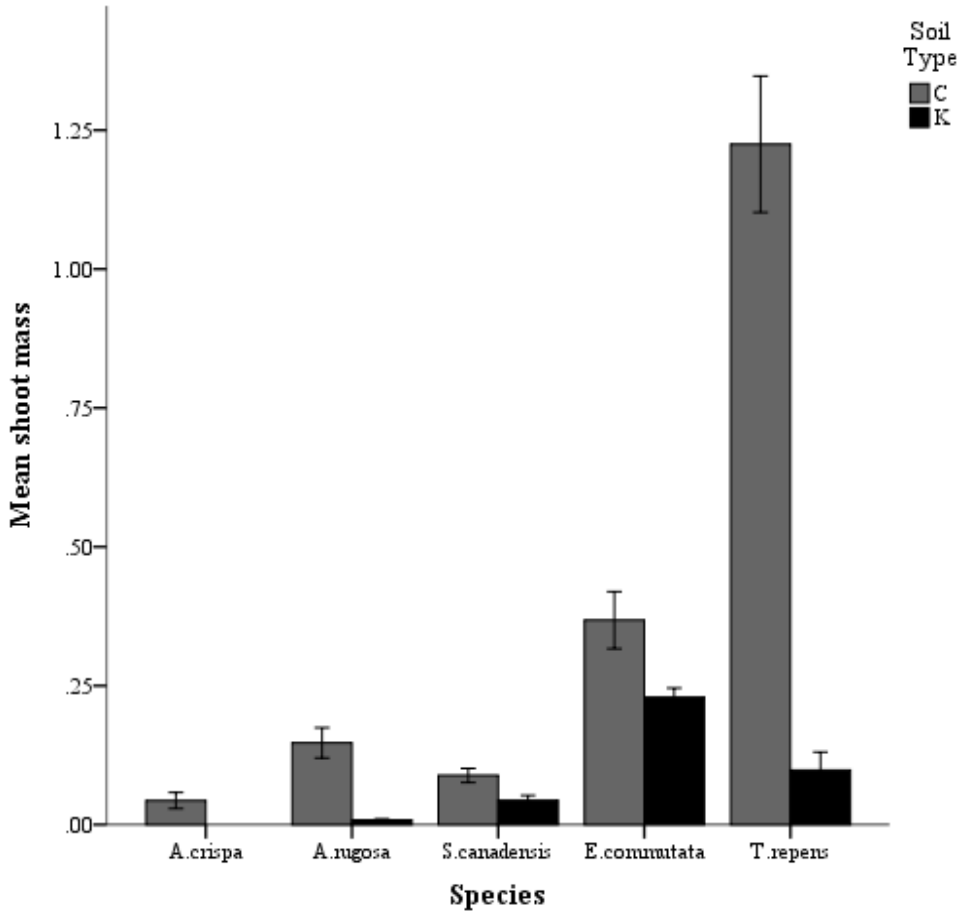

Figure 3. Effect of plant species and soil type (C-control; K-kimberlite) on dry shoot mass (g). Standard error bars represent +/- one standard error. Raw data displayed, but square root transformed for analysis.

Root to Shoot Ratio

The mean root to shoot ratio was slightly but significantly higher for the plants grown in the kimberlite soil compared to the control ($P<0.001$, Table 4, Fig. 4). This time, however, there was no significant differences in root to shoot ratio due to species ($P=0.497$) and soil inoculation methods ($P=0.733$). There was a borderline interaction between soil type and species ($P=0.052$), because *A. rugosa* had much higher root to shoot ratio on kimberlite soils than in the vermiculite control. All other interactions were insignificant ($P>>0.05$). *A. rugosa* had the largest difference in mean root to shoot ratio for plants grown in kimberlite compared to control soils (Fig. 5).

Table 4. Factorial ANOVA for plant root to shoot ratio. $P < 0.05$.

Source	df	MS	F	P
Inoculant	1	0.007	0.1	0.733
SoilType	1	0.895	14.3	**<0.001**
Species	4	0.054	0.9	0.497
Block	4	0.104	1.7	0.171
SoilType * Species	3	0.171	2.7	**0.052**
Inoculant * SoilType	1	0.004	0.06	0.804
Inoculant * Species	4	0.023	0.4	0.833
Inoculant * SoilType * Species	3	0.016	0.3	0.860
Error	63	0.063		

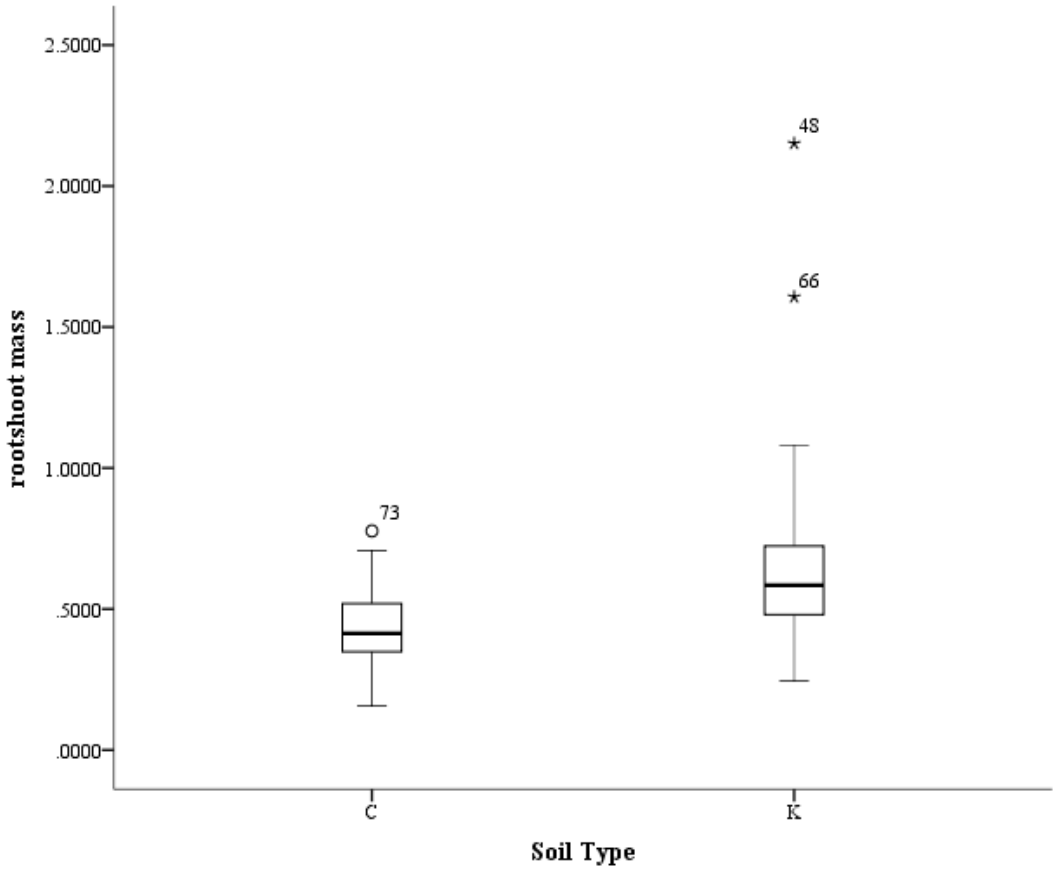

Figure 4. Effect of soil type (C-control; K-kimberlite) on plant root to shoot ratio.

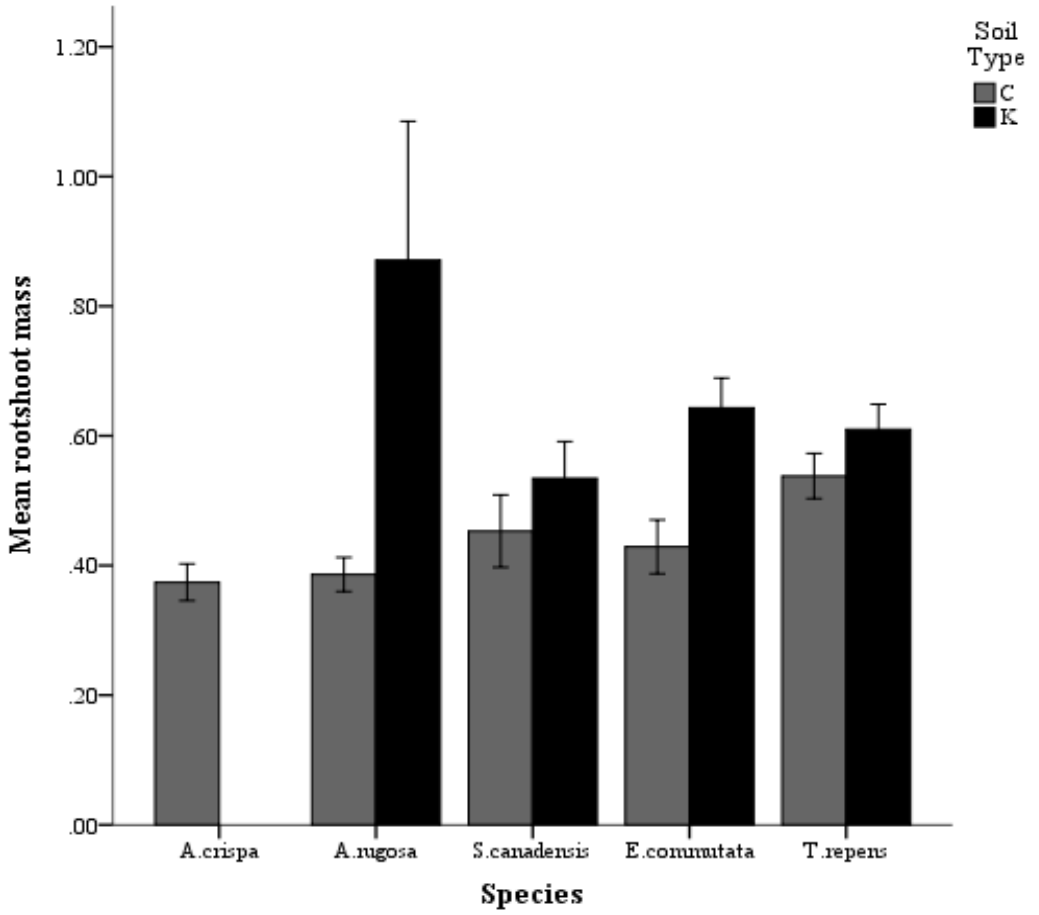

Figure 5. Effect of plant species and soil type (C-control; K-kimberlite) on the root to shoot ratio. Standard error bars represent +/- one standard error.

Nodulation

The data was square root transformed to meet the assumptions of the ANOVA. It also showed a similar pattern to the other growth parameters. The mean number of nodules per plant was significantly different for plant species and soil medium ($P<0.001$, Table 5), but not for soil inoculation methods ($P=0.171$). The greatest number of nodules was found on *T. repens* (8.72), followed by *A. rugosa* (2.71) *and A. crispa* (1.76) which were considered statistically similar. The lowest number of nodules were found on *S. canadensis* (0.99) *and E. commutata* (0.96) and were not considered significantly different ($P>>0.05$, Fig. 6). On average, there was more nodulation on plants in the control soil, but this was not universal and was detected as a

significant interaction (P<0.001); *S. canadensis* and *E. commutata* had the opposite trends with similar or more nodulation on the kimberlite soil than in the control soil.

Table 5. Factorial ANOVA for square root transformed nodules per plant. P <0.05.

Source	df	MS	F	P
Block	4	0.286	0.1	0.968
Inoculant	1	4.032	1.9	0.171
SoilType	1	69.809	33.3	**<0.001**
Species	4	198.000	94.5	**<0.001**
SoilType * Species	3	45.823	21.8	**<0.001**
Inoculant * SoilType	1	1.510	0.7	0.400
Inoculant * Species	4	1.677	0.8	0.530
Inoculant * SoilType * Species	3	4.222	2.0	0.121
Error	63	2.098		

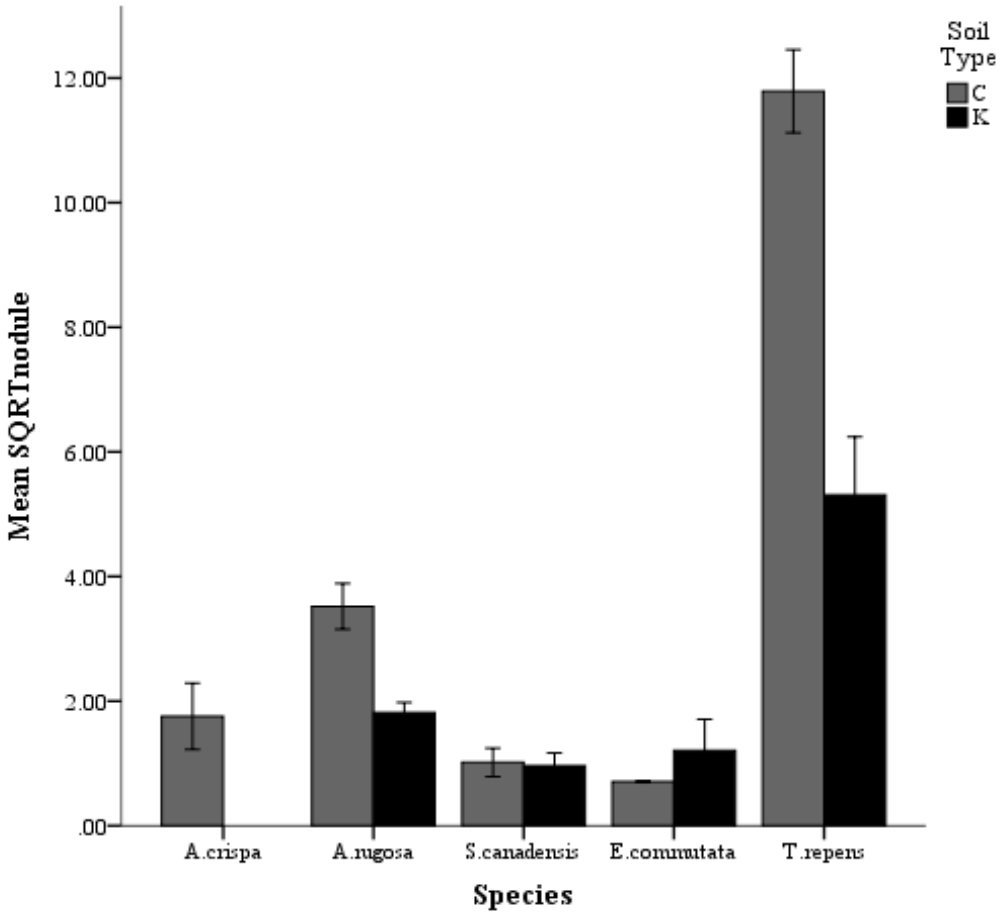

Figure 6. Effect of plant species and soil type (C-control; K-kimberlite) on the mean square rooted nodules per plant. Standard error bars represent +/- one standard error.

The mean pH of the control and kimberlite soil was 6.03 and 8.23 respectively (Table 6).

Table 6. pH calculated values for control and kimberlite soils.

				Mean pH
Control/Vermiculite	5.97	5.84	6.53	**6.03**
20 60 20/Kimberlite	8.03	8.39	8.37	**8.23**

DISCUSSION

The 20:60:20 kimberlite soil amendment showed the greatest growth potential for *T. repens* and *Poa pratensis* in experiments by Rouble (2011) and Bergeron (Laurentian Msc candidate, unpublished). It was expected in this study that the kimberlite soil would support plant growth but with a lower total biomass when compared to the control (Rouble 2011; Bergeron Laurentian Msc candidate, unpublished; Proctor 1971; Clarkson 1965). As expected, the control soil supported a higher plant biomass and lower root to shoot ratios compared to the 20:60:20 (FPK:CPK:silt) mineral soil. This may have to do with both physical and chemical aspects of the soils. Although we did not determine the detailed chemistry of both soils in this experiment, we confirmed the high pH of the kimberlite soil (8.23) compared to the control (6.03) with results by Bergeron (Laurentian Msc candidate, unpublished) and Rouble (2011). In addition we were able to refer to the detailed analysis made by Bergeron (Laurentian Msc candidate, unpublished) and Rouble (2011), for water retention, individual soil particle sizes, and elemental composition of each material individually.

The kimberlite soils were found to have a low Ca:Mg (<1), similar to serpentine soils that has is problematic for plant growth (Brady *et al* 2005), however with the addition of silt (crushed limestone) and peat to the soil amendment, the Ca:Mg should increase, and alleviate this issue (Rouble 2011).

At a high pH, such as in the kimberlite soil, iron may be limited or unavailable (Brown & Jolley 1989), therefore *E. commutata* and *S. canadensis* have an advantage due to their larger seed size storage, compared to *Alnus* sp. and *T. repens*. According to Bergeron (Laurentian Msc candidate, unpublished), the total nitrogen in the soil is minimal, the peat contains the only

source of total nitrogen, but it is not readily available for plant use; Again, this creates an advantage for *E. commutata* and *S. canadensis* in the initial growth stages due to large seed size. To support this point of discussion, I did observed nutrient deficiencies, including chlorosis in *T. repens* and browning of the tips and chlorosis on leaves of *A. rugosa* in kimberlite soils, however no signs of deficiency were visible in *E. commutata* and *S. canadensis*.

I also observed cracks in the kimberlite soil as a result of the fine textured soils, indicating probable differences of moisture, porosity and root penetration as compared to the vermiculite control. The fine textured soils: FPK and silt, beneficially increase CEC and water holding capacity of the substrate while aggregating the soil, however, also reduce soil pore space (Bergeron, Laurentian Msc candidate, unpublished). The CPK provides soil structure and adds pore space to the soil, which is essential for root development and nodulation (Dommergues 1997). The addition of peat to the soil, like the fine particle soils help to increase CEC, water holding capacity, soil aggregation, and adds organic material and pore space (Bergeron, Laurentian Msc candidate, unpublished). This increasing peat content was shown to be positively correlated with an increase in plant growth in other soils made from kimberlite (Reid & Naeth 2005; Rouble 2011). Fine texture can make plant establishment impossible for some species (Rajakaruna 2009). The reduction in pore space makes root development more difficult and nodulation more challenging (Dommergues 1997). In addition, needle ice formation in fine textured soils compared to coarser textured soils results in greater disturbance of root system caused by lifting of roots (Brink *et al* 1964). The soil amendment I used contains large sized CPK and should help to reduce negative effects of needle ice.

The differences in biomass between plant species was expected due to the inherent differences in their growth rates and lifecycles. *T. repens* is faster growing in comparison to

these shrub species, however ultimately it does not reach as large a size; this results in different sizes of plants at the end of the six week growth period. As well Elaeagnaceae have larger seeds compared to *T. repens* and both *Alnus* sp. and so the plants were larger upon germination. *T. repens* was expected to have the largest growth in the six week period. *Trifolium* was used in this experiment as a control species, because it is known for: early colonization at disturbed sites, tolerance to disturbance (Jiang-Wen *et al* 2003), successful growth on serpentine type soils (Moore & Zimmerman 1977), and its ability to perform nitrogen fixation. There are, however, native legumes in HBL, including: *Vicia americana* and *Lathyrus palustris* (Riley 2003), that were not used because seeds were not available. The native legumes could prove to be interesting additional plants for re-vegetation if seed could be obtained. The seed source and origin can dramatically influence plant success and establishment (Brady *et al* 2005). Therefore the native legumes may have responded differently than *T. repens* to growth in kimberlite soils. In the case of serpentine soils, seeds taken from strains of non-serpentine soils were either unsuccessful in establishing or significantly stunted on serpentine soils (Brady *et al* 2005). This should be taken into consideration when selecting seeds for reclamation. Legumes in general colonize quickly and have prostrate growth on barren soils, therefore will better help to prevent soil erosion than shrubs initially (Panciera & Sparrow 1995). In some cases legumes such as lupines, have been shown to contribute much more to soil nitrogen and to increasing nitrogen availability for non N-fixing plants compared to alders (Myrold & Huss-Danell 2003). Therefore although native legumes were not used in this experiment, they should be considered in combination with actinorhizal shrubs to maximize benefits of reclamation and increase diversity of N-fixing plants.

Interestingly, in our experiment, *S. canadensis* and *E. commutata* had the smallest differences in mean biomasses and number of nodules between kimberlite and control soils compared to *T. repens* and *A. rugosa* that had significantly reduced biomasses and number of nodules per plant in kimberlite soil when compared to the control soil.

Alders are commonly used in reclamation projects in Canada and around the world with varying levels of successful establishment (Roy *et al* 2007; Robb 2001). This experiment is one of many to test the success of alder growth on mine soil waste or soils affected by mining activity (Robb 2001; Roy *et al* 2007) and determining its reclamation ability. *Alnus* sp. have been successful in establishing in soils with a high pH of 12.1 (Chatarpaul *et al* 1990; Roy *et al* 2007) and were expected to have successful growth in kimberlite soils, however, generally both *Alnus* sp. have shown optimal growth at a pH of approximately 5.5 to 8.0, with optimal nodulation and shoot growth closer to 5.5 (Berry & Torrey 1986). In an experiment by Wheeler *et al* (1981) the optimal pH for *Alnus* sp. was approximately 4.5 to 6.5 and according to Schalin (1968) *A. crispa* prefers a pH closer to 4. In contrast, species of Elaeagnaceae are more commonly found in areas with a higher pH compared to *Alnus* sp. (USDA 2012). In an experiment by Zitzer & Dawson (1992) *Frankia* strains from soils with a pH 6.6 and higher were successful at nodulation of *Elaeagnus angustifolia*, whereas *Frankia* strains from soils with a lower pH of approximately 4.9, were the most successful at nodulating *Alnus glutinosa.* It is possible that the high pH of the soil was related to poor growth of *Alnus* sp. in kimberlite soils and the interactions with soil type. In addition, the low available nitrogen of the soil increases the need for immediate nodulation, especially in *Alnus* sp. and *T. repens* (again due to the small size of the seed). However, while nodulation provides the plant with the required nitrogen, it is energetically costly and has been shown to dramatically reduce the quantity of carbon allocated

to growth (Larue & Patterson 1981), in the case of legumes, non-nodulated plants were shown to contribute 810mg CO_2 to growth compared to nodulated individuals in the same conditions that could only allocate 510mg CO_2 to growth (Larue & Patterson 1981). Furthermore, the minimal pore space (Dommergues 1997) and high pH (Zitzer & Dawson 1992) in the kimberlite soil is a likely cause of reduced nodulation of *T. repens* and *A. rugosa* from control to kimberlite soil. The unfavourable pH, the reduction of nodulation in kimberlite soils, and the energetic cost of nodulation likely all played a role in the total mass reduction of *T. repens* and *A. rugosa* in kimberlite soils.

Elaeagnus sp. are widely used in Russia, Europe, and Asia (Kiseleva & Chindyaeva 2011), but its use for reclamation in Canada is not common. The species Russian Olive (*Elaeagnus angustifolia*) a non-native species to Canada and has received much more attention for its use in aesthetics and reclamation. *E. angustifolia* was shown capable to fix approximately 300kg N/ ha/yr after 3 years of growth (Khamzina *et al* 2009). Our study species *E. commutata* is however native to HBL and commonly found on frequently disturbed shorelines in the HBL (Riley 2003). In our experiment, *E. commutata* had significantly greater total mass compared to the other three shrub species, despite the low mean number of nodules formed. *E. commutata* and *S. canadensis* grown in each kimberlite and control soil had more similar masses, number of root nodules, and root to shoot ratios compared to both *Alnus* sp. and *T. repens* in our experiment. This suggests that the stressful soil properties of the kimberlite soil does not impede their growth or nodule formation and is rather similar to that of the control, making them good candidates for reclamation at Victor Mine. *Elaeagnus* sp. can be long lived up to 100 years (Kiseleva & Chindyaeva 2011) and have also been observed as colonizers of disturbed slopes (Moore 1964), therefore in the climate of the HBL are a very suitable species, such that they

require little maintenance once established and can be long lived while supplementing nitrogen to the soil. *S. canadensis* and *E. commutata* may be good candidate species for growth in the field because of the small differences in mass, nodule development, and root to shoot ratio in control and kimberlite soils.

A. crispa did not successfully establish in any kimberlite soil treatment. This in comparison to other species in the study may have been a result of the size of the plant at transplant time and/ or shock from the transplant to kimberlite soil with a high pH (Schalin 1968). Due to the small size of the plant, transplantation was more difficult, which may have caused damage to the plant tissue and root desiccation. In comparison the seedlings of the other three shrubs were much larger when their true leaves were developed. I also observed that it was the establishment of the seedling in the soil that was difficult, suggesting it may have been a consequence of transplanting methods as well a result of the soil conditions for *A. crispa*. Robb (2001) had a challenging time with establishment and successful germination of *A. crispa* for several reasons including contamination by fungus, algal growth, etc, which may have also affected the establishment of *A. crispa* in this experiment.

The two inoculation methods did not result in significant differences in the number of nodules per plant, differences in biomass, nor was any general trend observed. The number of nodules found on *S. canadensis* and *E. commutata* was significantly lower than *A. crispa*, *A. rugosa*, and *T. repens*. However, the number of nodules did not differ greatly between soil mediums for *S. canadensis* and *E. commutata*. These species were at a disadvantage because the lack of available local resources needed to create the inoculation materials. Therefore leaf litter and root material from *S. canadensis* was added to the litter tea however no root nodules from either plant species were available. *Myrica gale* litter and nodules and litter were added to the

appropriately inoculants, because they have been shown capable of nodulating Elaeagnaceae, despite being from different families (Huguet *et al* 2001). *S. canadensis* and *E. commutata* did not appear to have any visible mycorrhizal formation after 6 weeks of growth. *A. crispa*, *A. rugosa*, and *T. repens* showed fine root development which may have been a result of symbioses with a mycorrhizal species. Although the method of inoculation in this experiment did not demonstrate different effects of plant growth or nodulation, inoculation is essential for actinorhizal shrubs before transplant into soils made from mine waste rocks. Although there is evidence that some *Frankia* spores can persist in early successional sites that previously had no actinorhizal hosts, the chances are reduced (McCray Batzli *et al* 2004) and the site is referred to as having low inoculum potential (Hutton *et al* 1997). In mining soils and peatlands the amount of *Frankia* is limited (Seeds & Bishop 2009; Huss-Danell & Frey 1986) especially after disturbance and stockpiling (Hutton *et al* 1997). Because the mine waste rock at Victor mine was extracted from many meters beneath the surface and then stockpiled, the inoculum potential is further reduced (Hutton *et al* 1997).

CONCLUSION

The soil properties of the kimberlite soils, such as alkaline pH, low organic material, nitrogen, nutrients, and small particle size, poses obstacles for plant growth. However, nitrogen fixing shrubs are known for their tolerance to stress or disturbance, such as rocky lake shores or mine tailings and ability to colonize soils with almost no available nutrients. *E. commutata* and *S. canadensis* showed the greatest potential for remediation of Victor mine waste materials due to the small differences in biomass and nodule formation in the kimberlite and control soils. The biomass and nodulation was significantly reduced for *A. rugosa* grown in the kimberlite soils, likely due to the high pH and small seed size. *A. crispa* was unsuccessfully transplanted into kimberlite soils and all resulted in fatality shortly after. *E. commutata* and *S. canadensis* are native to the HBL and were successfully grown in kimberlite soils in a growth chamber at optimum temperatures. Native legumes species should be planted in conjunction with *E. commutata* and *S. canadensis* to increase soil nitrogen inputs and reduce soil erosion of new uplands. The next step is transplant into the field following inoculation using nodules and litter from the same species found in the region.

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