Proceedings of the 21st Annual British Columbia Mine Reclamation Symposium in Cranbrook, BC, 1997.
The Technical and Research Committee on Reclamation

ESTABLISHMENT AND GROWTH OF MYCORRHIZAL AND *RHIZOBIUM* INOCULATED HIGH-ELEVATION NATIVE LEGUMES ON AN UNAMENDED COAL MINE SPOBL DUMP IN SOUTHEASTERN BRITISH COLUMBIA.

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ABSTRACT

Direct seeding, transplant survivorship, growth and reproductive performance of seven mycorrhizal and *Rhizobium* inoculated high-elevation native legume species were studied at a coal mine in the Rocky Mountains of southeastern British Columbia.

Mycorrhizal and *Rhizobium* infection levels were low in the control treatment and highest in the combined inoculation treatments of all species. Transplant survival of each species was greatest for the combined inoculation treatment and lowest for the uninoculated control.

Inoculation with mycorrhizae, *Rhizobium* or a combination of mycorrhizae and *Rhizobium* resulted in greater mean plant diameters, mean plant heights, mean numbers of leaves per plant and mean percentage flowering than the uninoculated control plants. Measured plant parameters for the single inoculation treatments, i.e., mycorrhizal or *Rhizobium,* were greater than the control, but the greatest increases were recorded for the combination inoculation treatment. The greatest differences in the measured parameters were recorded for silky locoweed *(Oxytropis sericea* Nutt.) although differences were also large for Bourgeau's milk-vetch *(Astragalus bourgovii* A. Gray), Robin's milk-vetch *(Astragalus robbinsii* [Oakes] A. Gray) and bent-flowered milk-vetch *(Astragalus vexilliflexus* var. *nubilus* Barneby).

The results of the study have important management implications for the successful establishment of these species on unamended spoil dumps at high elevations.

INTRODUCTION

Agronomic legume establishment is problematic for high-elevation disturbances (Errington 1979, Johnson and Rumbaugh 1986, Smreciu 1993). Legumes or other species possessing a di-nitrogen fixation symbiosis have important ecosystem functions which can increase the nitrogen capital of the soil (Brown and Chambers 1990, Kenny and Cuany 1990) and stimulate biological soil activity (Flueler and Hasler 1990). Furthermore, legumes are important because of high levels of crude protein, vitamins and minerals (phosphorous and calcium) which make it valuable for forage and for di-nitrogen fixation (Klebesadel 1971). Grasses grown with legumes may benefit also from the nitrogen 'fixed' by legumes through re-cycling of decomposition and comminution of legume plant material (Chapin 1983).

Success or failure of legume establishment on mine spoil is dependent upon several biotic and abiotic factors, not the least of which, is the legume - *Rhizobium* symbiotic system (Skousen 1986). The effective nodulation by appropriate *Rhizobium* species is essential (Somasegaran and Hoben 1994). Since mine spoil as a growing medium for plants is very infertile, three important factors must be considered: (1) what is the rhizosphere colonizing ability of a particular strain of *Rhizobium,* (2) how effective are the resultant nodules and (3) how does inoculation compare with the host plants' response to soil nitrogen.

As well, mycorrhizal symbioses are known to enhance seedling establishment and survival (Allen and Allen 1980, Lambert and Cole 1980, Allen 1984, Allen et al. 1987, Waaland and Allen 1987) and plant growth (Hayman 1986, Azcôn-Aquilar and Barea 1992) through increased uptake of phosphorous and improved water relations or drought resistance (Allen and Friese 1992). The primary benefit of mycorrhizae is improved uptake of slowly diffusing ions of macronutrients such as phosphorous and

micronutrients such as zinc and copper (St. John 1990). Mycorrhizal symbionts are known to improve significantly inorganic nutrient uptake and growth in plants of boreal and alpine habitats (Mullen and Schmidt 1993).

Mycorrhizal infection is also generally best developed under conditions of nutrient stress (Smith and Read 1996). In legumes, the efficiency of phosphorous nutrition appears to stimulate nodule production and increase the rate of atmospheric nitrogen fixation (Barea and Azcón-Aquilar 1983, Barea et al. 1989). Authors studying *Hedysarum boreale* Nutt., a similar legume species to those of the present study, have indicated dependence of that species upon the mycorrhizal relationship for growth and survival (Reeves et al. 1979, Redente and Reeves 1981). Many of the species of this study have similar attributes and the potential for mycorrhizal infection is high. Therefore, inoculation of known mycorrhizal species may be a pre-requisite for establishment of these species on the low nutrient and moisture status spoil materials of waste rock dumps.

RESEARCH OBJECTIVES

The objectives of the study were to assess the efficacy of *Rhizobium* and mycorrhizal inoculation of a select group of native high-elevation legumes under growth chamber, glasshouse and field (mine spoil) conditions.

THE STUDY AREA

Based on the biogeoclimatic classification system used in British Columbia (Pojar et al. 1987), the study area for the Line Creek was located within the Engelmann Spruce - Subalpine Fir (ESSF) zone. The climate is characterized by short, cool growing seasons and long cold winters. The field experiments were positioned on a 5° west-facing slope of a dump platform (1930 m A.S.L.) dump platform. All research trials were established on unamended spoil. Composite (n = 5) spoil samples were withdrawn from each site, and their physical and chemical (nutritional) properties *analyzed* (Table 1). The spoils were composed primarily of siltstone and sandstone. In general, the spoils were high in percent coarse fragment contents (>2 mm) and percent sand (grain size <2 mm) and low in total nitrogen and available phosphorous.

Table 1. Spoil characteristics at the Line Creek Mine 1930m dump.

Parameter	Value
Coarse Fragments (%>2 mm)	64.00
Grain Size (<2mm)	
Clay (%)	1.02
Silt (%)	2.89
Sand (%)	96.09
pH ($CaCl_2$)	7.5
C_{org} (%)	3.57
N (%)	0.17
C:N	21.0
S (%)	0.05
CEC (meq/100g)	14.76
Ca (meq/100g)	12.56
Na (meq/100g)	0.44
Mg (meq/100g)	3.39
K (meq/100g)	0.34
P (ppm)	7.85

At each location, spoil surface and interstitial resistances were checked with a hand held penetrometer and were found to be acceptable for seeding or transplanting.

MATERIALS AND METHODS

Species Selection

Seven high elevation native legumes were selected for experimentation based on the research of Bell and Smyth (1988): alpine milkvetch *(Astragalus alpinus* L.), Bourgeau's milk-vetch *(Astragalus bourgovii* A. Gray), Robin's milk-vetch *(Astragalus robbinsii* [Oakes] A. Gray), bent-flowered milk-vetch *(Astragalus vexilliflexus* var. *nubilus* Barneby), stalked-pod crazyweed *(Oxytropis podocarpa* A. Gray.) and silky locoweed *(Oxytropis sericea* Nutt.).

Seed Collection and Sampling

Seeds of each legume species were hand-collected from subalpine and/or alpine plant populations (metapopulations) located on selected mountains adjacent to each transplant location. All seeds were stored in paper bags at room temperature until extraction and cleaning. Once processed, the seeds were stored in plastic bags in a refrigerator at 2-5°C (Young and Young 1986).

Seeds from a single accession of each species were withdrawn randomly with a Boerner Separator (Copeland and McDonald 1985). Single accessions were chosen to reduce within-species transplant survival variability. Accession selection was restricted to populations for which a large quantity of seed had been collected (N>1000). Seeds of each species were x-rayed for two minutes in a Faxitron 43855a X-Ray Unit set at 15 KVP (Smyth 1987). Pure Live Seed (PLS) were removed following examination of the x-rays. The seeds were surface sterilized in 4% Ca(OCl)2 for 10 minutes, rinsed five times in sterile water, soaked in 95% ethanol for 5 minutes and then rinsed a further five times (Garvin and Lindemann 1983). Seeds which appeared undamaged after the sterilization process were then scarified (single cut with sterilized scalpel).

Rhizobium Inoculation - Greenhouse

A four treatment randomized-block design experiment with three replicates (n=100) was performed to examine the efficacy of indigenous strain *Rhizobium* inoculation on container seedlings. The four treatments were as follows: (1) control - uninoculated seedlings and fertilizer minus nitrogen, (2) uninoculated seedlings and fertilizer plus nitrogen, (3) *Rhizobium* inoculation and fertilizer minus nitrogen and (4) *Rhizobium* inoculation and fertilizer plus nitrogen. A randomized block design (Little and Hills 1978) was employed.

Seedling production involved several stages. Cone-Tainers™ (depth - 16.3 cm, volume - 65.5 cm^3) were surface sterilized with 2.5% NaOCl and rinsed three times in sterile water. Sterilized (autoclaved) growth medium of 2:1:1 (peat, vermiculite and perlite) was prepared and placed within the container cells. Two seeds per cone were placed on top of the soil mix and covered with one centimeter of sterilized forestry grit. The soil mix was then saturated with sterile Hoagland's solution (Hoagland and Broyer 1936) with or without nitrogen.

The plants were inoculated with appropriate *Rhizobium* strains (liquid culture) after the cotyledons had cleared the surface of the vermiculite. Inoculation was delayed until after the emergence of the seedlings in order to eliminate the possibility of contamination. The cones were also covered with aluminum foil to prevent contamination during the first days of germination. Subsequent waterings alternated between sterile water and sterile Hoagland's solution (Garvin and Lindemann 1983). The plants were grown in a greenhouse until harvest after 4 months of growth (Gibson 1980). In the harvesting process, the Cone-Tainers™ were inverted and the root system washed in a bucket of water to remove the growth media. The roots were then examined carefully.

The respective *Rhizobium* cultures were grown in shake flasks of yeast extract-mannitol (YEM) broth for two weeks. An approximate initial population of 100 million rhizobia per inoculum bag was obtained in

order to ensure inoculation success. Rhizobium inoculum populations were estimated with the Most Probable Number (MPN) method of Vincent (1970). Equal volumes of liquid inoculum and sterile peat were mixed aseptically in whirl-pak bags and incubated at room temperature for 7 days, following which, the inoculum was stored in a refrigerator at 5°C until inoculation. The sources for the initial inoculum culture were fresh nodules from each species obtained during the previous summer.

The data recorded was as follows: number nodulated, nodule location and plant growth. The distribution codes of Allen and Allen (1981) were used to classify species nodulation (Table 2).

Table 2. Nodulation distribution classification[a].

Nodule Score	Distribution and Number of Effective Nodules[b]	
	Crown[c]	Elsewhere
0	0	0
½	0	1-4
1	0	5-9
1½	0	>10
2	Few	0
2½	Few	Few
3	Many	0
4	Many	Few
5	Many	Many

[a] After Allen and Allen (1981)
[b] Effectiveness judged on basis of nodule size and internal pigmentation; ineffective nodules not considered.
[c] Crown regarded as the top 5 cm of root system

Rhizobium Inoculation - Mine Spoil

Commercial peat was purchased from the Nitragin Incorporated (Milwaukee, Wisconsin), divided into seven portions and sterilized in separate autoclavable bags. The peat in each bag was then re-inoculated with an indigenous strain of *Rhizobium* specific to each legume species. Ten fifty seed replicates which contained surface sterilized (refer to preceding section) seeds of each species were then coated with their respective strain of peat inoculum. Five replicates of each species were also coated with sterile non-inoculated peat. The replicates were then sown in a completely randomized block design at Line Creek during early spring. Seed was sown with an aluminum hand seeder which was sterilized between replicates with 70% ethanol. The developing seedling received weekly liquid fertilizer applications of Hoagland's solution (Hoagland and Broyer 1936). Five randomly selected replicates of inoculated seed of each species received a nitrogen Hoagland's solution treatment. Seedlings were monitored for growth and vigor. Seedlings growth and vigor were assessed visually and after two growing seasons, 100 plants per species and treatment were excavated to determine nodulation and infectiveness.

The data recorded was as follows: number nodulated, nodule location and plant growth. The distribution codes of Allen and Allen (1981) were used to classify species nodulation (Table 2).

Mycorrhizal Relationships

Root samples were collected from three populations within the designated study area of the Southeast Kootenay Coal Block. Root samples were obtained by excavating five plants, carefully removing the adhering soil from the roots and excising 10 root segments from each plant. The root segments, 10-20 mm in length were then washed with distilled water, cleared stained and mounted in glycerine jelly (Phillips and Hayman 1970). All samples were then examined with a compound microscope to indicate presence or absence of fungal infection. No attempt was made to identify the fungus association or

quantify the amount of root infection. The fungal associations were classified according to the definitions presented in Moore-Landecker (1982).

Mycorrhizal Inoculation

A four factor experiment was performed to examine the effects of fungal associations on the survival, growth and reproductive activity of the legume species. The various factors were as follows: (1) control - 1:2 ratio (volume) sterilized quartz sand and sterilized soil, (2) plus *Rhizobium* treatment - 1:2 ratio sterilized quartz sand and sterilized soil plus *Rhizobium* inoculum, (3) plus fungi treatment - 1:2 ratio sterilized quartz sand and soil, and (4) + fungi and *Rhizobium* treatment -1:2 ratio sterilized quartz sand and soil. The soil for the plus fungi treatment was obtained by sterilizing an appropriate field soil for each species and then re-inoculating with spores and hyphae generated from greenhouse host "trap" plants (Schenck 1982). Two hundred plants per species and treatment were grown in sterilized Cone-Tainers™ in the greenhouse for four months. The seedlings were then randomly assigned and transplanted into rows within blocks on a leveled area of the 1930 m dump. The seedlings were spaced twenty-five centimeters apart (Brockwell et al. 1982) within each block. Each block was separated by a one meter pathway. Dormant seedlings were transplanted by shovel shortly after snow-melt in the spring. Only healthy and nodulated seedlings were transplanted.

Appropriate indigenous *Rhizobium* inocula were used for each species. All transplants were checked for viable *Rhizobium* nodulation prior to transplantation. As well, twenty-five plants of each species per treatment were randomly selected for destructive examination of fungal infection assessment prior to transplanting (Hayman et al. 1981). Plant height (cm), plant diameter, number of leaves, plant survival and flowering data was recorded after two years of growth. One hundred plants per species and treatment were randomly selected and excavated for assessment of mycorrhizal and *Rhizobium* infection at the termination of the field experiment. The roots were examined following the procedures of Phillips and Hayman (1970) and classified according to the criteria of Carpenter and Allen (1988). No attempt was made to identify the fungus association or quantify the amount of root infection.

RESULTS

Rhizobium Inoculation - Greenhouse

The growth chamber results are listed in Tables 3 and 4. The number of nodules per ranged from a mean of 9.2 for *Hedysarum sulphurescens* to 28.5 *for Astragalus robbinsii.* The percentage of effective nodules ranged from 57.8 for *Astragalus vexilliflexus* var. *nubilus* to 75.2 for *Hedysarum sulphurescens.* The distribution and number of effective nodules for each species was classified. Most of the nodules on each species were few in number and located on the secondary peripheral roots. *Astragalus alpinus* was an exception with several nodules located on both the crown as well as the peripheral root system.

Table 3. Classification of Nodulation of Selected Legumes.

Plant Species	Greenhouse	1930 m Dump
Astragalus alpinus	5	2½
Astragalus bourgovii	1½	1½
Astragalus robbinsii	2½	2½
Astragalus vexilliflexus	1	1
Hedysarum sulphurescens	2½	2½
Oxytropis podocarpa	1	1
Oxytropis sericea	2½	2½

Table 4. Nodulation of Containerized Seedlings in the Greenhouse and on the 1930 m Dump.

Plant Species	Number of Plants Sampled	Greenhouse		1930 m Dump	
		Number of Nodules / Plant	Percentage of Pink Nodules / Plant	Number of Nodules / Plant	Percentage of Pink Nodules/Plant
		Mean ± SE	Mean	Mean ± SE	Mean
Astragalus alpinus	100	18.8 ± 1.5	74.2	16.7 ± 3.7	80.9
Astragalus bourgovii	100	14.8 ± 1.7	68.7	14.5 ± 3.1	71.2
Astragalus robbinsii	100	28.5 ± 4.3	74.2	31.4 ± 2.9	85.3
Astragalus vexilliflexus	100	7.5 ± 2.3	57.8	18.1 ± 2.1	95.4
Hedysarum sulphurescens	100	9.2 ± 1.8	75.2	13.4 ± 1.7	82.1
Oxytropis podocarpa	100	9.4 ± 2.7	69.2	8.2 ± 1.3	90.3
Oxytropis sericea	100	15.1 ± 2.1	70.1	30.3 ± 2.4	91.4

Rhizobium Inoculation - Mine Spoil

The mine spoil results are also listed in Tables 3 and 4. The number of nodules per plant ranged from a mean of 8.2 for *Oxytropis podocarpa* to 31.4 for *Astragalus robbinsii.* The percentage of effective nodules for each species ranged from 71.2 *for Astragalus bourgovii* to 95.4 *for Astragalus vexilliflexus* var. *nubilus.* Most of the nodules on each species were few in number and located on the secondary peripheral roots. In general, the number of effective nodules was greater on the mine spoil in comparison to the greenhouse.

Modulation was sporadic for the uninoculated control. *Astragalus alpinus* and *Astragalus robbinsii* were not nodulated, whereas nodulation and nodule effectiveness was variable for the remaining species. Modulation and nodule effectiveness was high. Plants *of Astragalus alpinus, Astragalus vexilliflexus* var. *nubilus* and *Oxytropis sericea* were all inoculated and possessed effective nodules. The remaining species were inoculated, but the nodules were frequently ineffective. All plants of the inoculated plus nitrogen treatment were inoculated, but the percentage of effective nodules per plant was variable.

Species growth and vigor was good or excellent for the inoculated and inoculated plus nitrogen treatments, but poor or fair for the uninoculated treatments (Table 5).

Mycorrhizal Relationships

All the selected species demonstrated some form of association with fungi. However, only *Hedysarum sulphurescens* was infected with vesicular arbuscular mycorrhizae. Mycorrhizal spore numbers among plant roots of *Hedysarum sulphurescens* was generally low although levels of root infection were high. External hyphae and often spores of the mycorrhizal fungi were common on the surfaces of all roots; however, mycorrhizal infections were only common on the smaller lateral roots. All stages of infection were observed: hyphal coils, simple hyphae, arbuscules and vesicles. Arbuscules were most frequent in the innermost cortical cells. Occasionally, primary and secondary lateral roots contained small amounts of minimally branched hyphae and scattered vesicles. VAM infection levels were qualitatively different between sample populations of *Hedysarum sulphurescens,* with infection levels greatest in the alpine populations. Evidence of dematiaceous septate fungi (DSF) was sporadic for the alpine populations of these species and non existent for the lower elevation populations.

Table 5. Nodulation of Containerized Seedlings in the Greenhouse and on the 1930 m Dump.

Plant Species	Treatment (n=100)	Greenhouse		1930 m Dump	
		Plant Nodulation	Growth	Plant Nodulation	Growth
Astragalus alpinus	- *Rhizobium*, - Nitrogen	-	Poor	-	Poor
	- *Rhizobium*, + Nitrogen	-	Fair	-	Fair
	+ *Rhizobium*, - Nitrogen	+E	Excellent	+, I or E	Excellent
	+ *Rhizobium*, + Nitrogen	+E	Excellent	+, I or E	Excellent
Astragalus bourgovii	- *Rhizobium*, - Nitrogen	+ I	Poor	+I	Poor
	- *Rhizobium*, + Nitrogen	+ I	Fair	+, I or E	Fair
	+ *Rhizobium*, - Nitrogen	+, I or E	Excellent	+, I or E	Good
	+ *Rhizobium*, + Nitrogen	+, I or E	Excellent	+, I or E	Excellent
Astragalus robbinsii	- *Rhizobium*, - Nitrogen	-	Poor	-	Poor
	- *Rhizobium*, + Nitrogen	-	Good	-	Poor
	+ *Rhizobium*, - Nitrogen	+E	Excellent	+E	Good
	+ *Rhizobium*, + Nitrogen	+, I or E	Excellent	+, I or E	Good
Astragalus vexilliflexus	- *Rhizobium*, - Nitrogen	-	Poor	+, I or E	Poor
	- *Rhizobium*, + Nitrogen	-	Good	+, I or E	Poor
	+ *Rhizobium*, - Nitrogen	+ E	Excellent	+E	Good
	+ *Rhizobium*, + Nitrogen	+, I or E	Excellent	+, I or E	Excellent
Hedysarum sulphurescens	- *Rhizobium*, - Nitrogen	-	Poor	- or + E	Poor
	- *Rhizobium*, + Nitrogen	-	Fair	- or + E	Poor
	+ *Rhizobium*, - Nitrogen	+, I or E	Good	+ I or E	Good
	+ *Rhizobium*, + Nitrogen	+E	Excellent	+E	Good
Oxytropis podocarpa	- *Rhizobium*, - Nitrogen	-	Poor	+, I or E	Poor
	- *Rhizobium*, + Nitrogen	-	Fair	+, I or E	Fair
	+ *Rhizobium*, - Nitrogen	+, I or E	Excellent	+, I or E	Excellent
	+ *Rhizobium*, + Nitrogen	+E	Excellent	+E	Excellent
Oxytropis sericea	- *Rhizobium*, - Nitrogen	-	Poor	- or + E	Poor
	- *Rhizobium*, + Nitrogen	-	Fair	- or + E	Fair
	+ *Rhizobium*, - Nitrogen	+, I or E	Good	+ I or E	Excellent
	+ *Rhizobium*, + Nitrogen	+E	Excellent	+E	Excellent

Key: (-) - plants not nodulated, (+) - plants nodulated, E - effective dinitrogen fixation, I - ineffective dinitrogen fixation
Plants may or may not be nodulated depending on their sensitivity to nodule formation being inhibited by applied nitrogen.

The *Astragalus* and *Oxytropis* species were only infected with dematiaceous septate fungi (DSF). The septate fungi were observed on the surface of the roots of these species and did not appear to have penetrated the root cortex. The walls of the hyphae were deeply melanized and septate. A number of clamp connections were observed as well. Clamp connections were more frequent with *Astragalus vexilliflexus* var. *nubilus* and *Oxytropis sericea.* All populations of the *Astragalus* and *Oxytropis* species were infected with (DSF), although there was an apparent increase in infection in populations found in higher elevation and exposed habitats. One hundred percent of the samples of collected for each of the *Astragalus* and *Oxytropis* species were associated with dematiaceous septate fungi.

Mycorrhizal Inoculation - Mine Spoil

Fungal and *Rhizobium* infection levels recorded during the final assessments were low for the control treatment for all species. The greatest fungal infections (Class 3) were recorded for the dual inoculation and fungal treatments although some of the transplants in the control and *Rhizobium* treatments had small amounts of fungal infections (Class 1 or 2) (Table 6).

Transplant survival of each species was greatest for the dual inoculation treatment and lowest for the uninoculated control. However, the differences in survival between treatments were not significant. One hundred percent of the dual inoculation treatment transplants of each species survived whereas, generally, one to two of the transplants of the single inoculation treatments died. All of the *Astragalus alpinus* transplants for each treatment survived.

Table 6. Fungal Infection Status for Each Species at the 1930 m Dump of Line Creek Mine.

Plant Species	Treatment	Classification			
		0	1	2	3
Astragalus alpinus	Control	89	7	4	-
	+ *Rhizobium*	87	5	4	4
	+ Fungi	20	13	46	21
	+ Fungi and *Rhizobium*	8	8	32	52
Astragalus bourgovii	Control	88	4	4	4
	+ *Rhizobium*	76	20	-	4
	+ Fungi	8	-	40	52
	+ Fungi and *Rhizobium*	4	4	32	60
Astragalus robbinsii	Control	96	4	-	-
	+ *Rhizobium*	95	5	-	-
	+ Fungi	11	-	39	50
	+ Fungi and *Rhizobium*	9	3	12	76
Astragalus vexilliflexus	Control	81	11	8	-
	+ *Rhizobium*	79	12	5	4
	+ Fungi	17	4	16	63
	+ Fungi and *Rhizobium*	4	8	8	80
Hedysarum sulphurescens	Control	83	17	-	-
	+ *Rhizobium*	80	11	9	-
	+ Fungi	16	3	36	45
	+ Fungi and *Rhizobium*	8	4	40	48
Oxytropis podocarpa	Control	96	4	-	-
	+ *Rhizobium*	89	4	7	-
	+ Fungi	17	7	24	52
	+ Fungi and *Rhizobium*	12	-	32	56
Oxytropis sericea	Control	91	5	4	-
	+ *Rhizobium*	96	-	4	-
	+ Fungi	24	4	4	68
	+ Fungi and *Rhizobium*	8	4	17	71

Inoculation with mycorrhizae, *Rhizobium* or a combination of mycorrhizae and *Rhizobium* resulted in greater mean plant diameters, mean plant heights and mean numbers of leaves per plant than the uninoculated control plants (Table *T).* Measured plant parameters for the single inoculation treatments were greater than the control, but the greatest increases were recorded for the dual inoculation treatment. The greatest differences in the measured parameters were recorded for *Oxytropis sericea* although differences for *Astragalus bourgovii, Astragalus robbinsii* and *Astragalus vexilliflexus* var. *nubilus* were also large. Total above ground biomass and leaflet drymass biomass measurements were not recorded because bighorn sheep *(Ovis canadensis)* had grazed on the plants following the recording of the preceding measurements.

Reproductive activity was also greatest for the dual inoculation although not significantly (Table 8). The control and the single inoculation treatments were very similar for all species except the *Oxytropis* species where there was a slight increase in the fungal inoculation treatment.

Table 7. Mean Plant Response to Fungal and *Rhizobial* Inoculation.

Plant Species	Treatment	Plant Parameters		
		Diameter (cm)	Height (cm)	Number of Leaves
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Astragalus alpinus	Control	11.6 ± 2.3	3.4 ± 0.6	12.7 ± 1.8
	+ *Rhizobium*	15.6 ± 2.1	5.6 ± 0.8	16.4 ± 1.5
	+ Fungi	14.5 ± 1.8	4.1 ± 1.1	17.3 ± 2.0
	+ Fungi + *Rhizobium*	19.3 ± 2.4	5.2 ± 1.3	30.5 ± 3.1
Astragalus bourgovii	Control	8.7 ± 1.3	3.5 ± 0.6	12.7 ± 1.5
	+ *Rhizobium*	10.2 ± 1.7	3.9 ± 0.8	20.8 ± 2.5
	+ Fungi	9.8 ± 1.7	3.6 ± 0.6	15.7 ± 1.9
	+ Fungi + *Rhizobium*	16.5 ± 1.3	4.2 ± 0.7	29.8 ± 3.9
Astragalus robbinsii	Control	7.8 ± 1.4	3.4 ± 0.6	7.4 ±1.1
	+ *Rhizobium*	10.7 ± 1.2	3.6 ± 1.0	13.7 ±1.7
	+ Fungi	8.9 ± 1.7	3.2 ± 1.1	12.8 ±2.0
	+ Fungi + *Rhizobium*	15.8 ± 2.0	5.5 ± 0.8	37.4 ±4.9
Astragalus vexilliflexus	Control	8.1 ± 2.0	0.6 ± 0.2	16.0 ±1.0
	+ *Rhizobium*	14.2 ± 4.0	1.4 ± 0.6	22.0 ±3.6
	+ Fungi	22.4 ± 0.9	1.7 ± 0.3	21.7 ±1.5
	+ Fungi + *Rhizobium*	32.3 ± 3.3	2.4 ± 0.2	33.7 ±7.4
Hedysarum sulphurescens	Control	7.1 ± 0.9	7.7 ± 0.6	6.8 ± 1.7
	+ Rhizobium	8.1 ± 0.8	8.4 ± 0.7	12.3 ± 3.0
	+ Fungi	10.8 ± 1.7	10.4 ± 0.9	9.4 ± 0.8
	+ Fungi + *Rhizobium*	16.0 ± 1.8	14.1 ± 2.4	16.8 ± 1.7
Oxytropis podocarpa	Control	5.1 ± 0.9	0.8 ± 0.2	16.7 ± 1.5
	+ *Rhizobium*	6.9 ± 0.7	1.0 ± 0.1	22.3 ± 1.5
	+ Fungi	6.1 ± 0.2	0.8 ± 0.1	18.3 ± 0.6
	+ Fungi + *Rhizobium*	8.9 ± 0.7	2.1 ± 0.3	41.3 ± 4.6
Oxytropis sericea	Control	8.6 ± 0.4	3.1 ± 0.4	11.3 ± 2.1
	+ *Rhizobium*	9.2 ± 1.6	3.9 ± 0.2	21.7 ± 1.2
	+ Fungi	8.9 ± 1.2	2.7 ± 0.2	19.0 ± 1.0
	+ Fungi + *Rhizobium*	15.5 ± 1.9	10.0 ± 1.4	36.0 ± 2.5

Table 8. Mean Plant Response to Fungal and *Rhizobial* Inoculation.

Plant Species	Treatment	Number of Individuals with Flowers or Fruit	Number of Individuals Lacking Flowers or Fruit
Astragalus alpinus	Control	11	89
	+ Rhizobium	15	85
	+ Fungi	16	84
	+ Fungi + Rhizobium	41	59
Astragalus bourgovii	Control	19	81
	+ Rhizobium	23	77
	+ Fungi	24	76
	+ Fungi + Rhizobium	61	39
Astragalus robbinsii	Control	16	84
	+ Rhizobium	19	81
	+ Fungi	20	80
	+ Fungi + Rhizobium	44	56
Astragalus vexilliflexus	Control	29	71
	+ Rhizobium	36	64
	+ Fungi	37	63
	+ Fungi + Rhizobium	62	38
Hedysarum sulphurescens	Control	12	88
	+ Rhizobium	17	83
	+ Fungi	17	83
	+ Fungi + Rhizobium	52	48
Oxytropis podocarpa	Control	16	84
	+ Rhizobium	17	83
	+ Fungi	32	68
	+ Fungi + Rhizobium	53	47
Oxytropis sericea	Control	19	81
	+ Rhizobium	23	77
	+ Fungi	44	56
	+ Fungi + Rhizobium	64	36

DISCUSSION

Rhizobium Inoculation - Greenhouse and Mine Spoil

Sporadic nodulation and poor legume growth is interpreted to indicate that the resident populations of indigenous *Rhizobia* in the mine spoil at the Line Creek mine are small. Only *Oxytropis sericea* occasionally possessed effective nodules. The number of nodules per plant was much lower than the numbers found on agronomic species such as *Medicago sativa* (Allen and Allen 1981). The small number of nodules may be an indication of a more efficient or conservative nitrogen metabolism for these legume species. Location of the nodules was generally in the peripheral zone rather than on the root crown. This is comparable to most agronomic species (Johnson and Rumbaugh 1981).

In general, there was a lower number of nodules and effective nodules per plant in the greenhouse experiment when compared to the mine spoil, a result observed by Johnson and Rumbaugh (1986). There did not appear to be any relationship between the number of nodules per plant and legume species. However, *Astragalus alpinus* is apparently more promiscuous than all the other species. All of the host plants appear to be sensitive to soil nitrogen levels, a physiological response which generally results in an increased number of ineffective nodules (Kenny and Cuany 1990).

Mycorrhizal Relationships

The fungal association results are in general agreement with that of Currah and van Dyk (1986). Most of the alpine species had extensive surface nets of dematiaceous septate fungi (DSF). Only Hedysarum sulphurescens was infected with vesicular arbuscular mycorrhizal. DSF infections of the *Hedysarum* species increased with an increase in elevation and exposure. The habitats of the alpine populations of all species were, in general, Regosols with small amounts of soil organic matter. The absence of VAM fungi in such soils has been documented in earlier research (Väre et al. 1992). Therefore, the present study confirms the results of previous studies as well as increases the number of known legume species which have either VAM or DSF associations.

Mycorrhizal Inoculation - Mine Spoil

The results of the fungal inoculation field experiment revealed that inoculation with either VAM or DSF fungi provides an advantage to the legume species. Survival, above ground biomass and reproductive activity were greater for the dual inoculation treatment of each species. Although yields were not quantified and detailed fertilizer trials were not conducted, it is expected that dual inoculation would result in the most favorable establishment results for these species. Inoculation with DSF and VAM fungi would also benefit the wide range of indigenous high elevation species that may be used in reclamation seed mixes.

The results of this section confirm published results for other species (Azcon-Aguilar et al. 1982, Redente and Reeves 1981, Skujins and Allen 1986, Carpenter and Allen 1988, Miller and Jastrow 1992). The production and inoculation *of Rhizobia* does not appear to present a serious problem from a operational perspective. However, mycorrhizal inoculation will be more difficult because of the fungal culturing problems and inoculation difficulties (Call and McKeII 1982). Nevertheless, attempts must be made to establish these microorganisms if successful establishment and survival of these legume species will be possible on high-elevation mine spoil.

ACKNOWLEDGMENTS

Funding for this project was provided by the Science Council of British Columbia, Fording Coal Limited - Fording River Operations and Line Creek Resources Limited.

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