

ESTABLISHMENT OF A TRIAL TO TEST DIRECT SEEDING OF LODGEPOLE PINE FOR RECLAMATION ON GIBRALTAR MINE PROPERTIES, BRITISH COLUMBIA

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ABSTRACT

Recent work with lodgepole pine has demonstrated that with the correct symbiotic partners, it can fix nitrogen which helps explain its pioneering nature. Current reclamation of disturbed sites to conifers employs the use of container stock and is not wholly successful. Problems with using nursery stock include seedling shock from harsh conditions associated with mine spoils and pre-colonization of seedlings by symbionts found in, and adapted to nursery conditions. Historically, direct seeding of pine in forestry in British Columbia putatively failed because of seed predation and desiccation. These challenges may be manageable and/or may not be important in reclamation work.

In the fall of 2011, a modified direct seeding trial using lodgepole pine was established on a rock dump covered with ½ meter of fine textured overburden at Gibraltar Mines in McLeese Lake, BC. *Pinus contorta* var. *latifolia* seed collected from the Gibraltar property had a laboratory germination success rate of 99% and was used to plant the 3 hectare trial. The site was mostly planted to bare seed with 4 14m x 14m sub-plots planted to various combinations of seed and symbionts. The theory, methods employed and experimental design of this trial and some of the challenges encountered are described.

KEY WORDS: Lodgepole pine, direct seeding, tuberculate mycorrhizae, microbial inoculation, mine reclamation

INTRODUCTION

An underlying principle of soil science, which Vasily Dokuchaev postulated in 1883, is that soil is a product of the interaction of parent material, climate, biota, geography and time. While all soil scientists and ecologists are familiar with this concept, not all of them grasp the profound implications that this principle has for reclamation work.

Broadly speaking soil in BC fits into three categories. Low elevation or grassland soils have a thin accumulation of litter on top of the soil and large quantities of organic matter intimately mixed with the surface mineral horizons. Lower elevation and well drained forests have relatively smaller amounts of organic matter intermixed with the surface mineral soil and moderate amounts of organic matter

accumulated on the surface. Higher elevation forest and wetland forests have deep accumulations of organic matter on the surface and even less organic matter incorporated in the mineral soil. The different types of plant communities associated with these different soil types are not only adapted to these soil types, but also play a role in the creation of the soil in which they dwell. There are also broad and characteristic patterns in the community of micro and meso soil organisms that occupy and constitute these different soil types (Read, 1991).

The concept that plant communities which develop on raw parent material will go through an evolutionary process or in ecological terms, seral stages, is widely accepted (Barbour et al., 2000). The soils in which those plant communities exist also go through an evolutionary process called pedogenesis. Because there is no inorganic source of mineral nitrogen in soil, one of most commonly identified soil evolutionary processes occurring as the plant community evolves is the accumulation of nitrogen capital in the parent material and subsequently in the developing soil. The accretion of nitrogen eventually allows non-nitrogen fixing species to occupy an area. Therefore, a first thought when trying to accelerate pedogenesis in mine spoils or other parent material is to create nitrogen capital by adding simple chemical nitrogen, more complex, typically organic sources of nitrogen, or nitrogen fixing species.

There are two main problems with this notion. The first is that while nitrogen accumulation is an important component of pedogenesis, it is only one of a myriad of changes that occur in the parent material as it is transformed into a soil that is capable of supporting a complex ecosystem. The second is that not all sources of nitrogen are equal. Environments in BC that tend to forest have higher (effective) precipitation than areas that tend to grass. Forest environments tend to have their nitrogen tied up in variable but relatively high C/N ratio organic matter from which nitrogen cannot be leached through the rooting zone and organic matter decomposition and nitrogen mineralization are both biologically mediated (Schmidt *et al.* 2011). Putting low C/N ratio organic matter into a wetter environment that tends to high C/N ratio organic matter means that neither organic matter nor nutrient will be conserved unless it is somehow quickly incorporated into high C/N ratio organic matter- an unlikely outcome when a site is being initially vegetated from parent material and if the predominant vegetation established on the site produces rapidly-decomposable litter, low C/N ratio litter. The facile solution to retaining nitrogen (and other nutrients) on site is to grow agronomic species which quickly establish into a dense cover and are capable of taking up a large proportion of any nitrogen or other nutrients added to the site. For many reasons, grassland or agricultural type soils are not the same kind of soils that sustain a forest and soils created by the addition of readily available nitrogen or easily decomposed organic matter, will not persist in an environment to which they are not adapted; and they will not evolve into forest soils without undergoing some major reorganization, such as the development of higher C/N ratio litter. Trying to maintain a grass cover in a forest environment ensures a never-ending struggle to try to maintain something that competition and environment work to eliminate. Left to its own devices, a forest will eventually overrun a grassland established in an environment suitable for forest development. In most cases agronomic type vegetation will delay rather than accelerate the process of establishing conditions suitable for a forest type community.

Establishing nitrogen fixing plants is another way to gradually increase the nitrogen capital on sites in the hopes of creating a more persistent cover. The commonly considered legumes do not produce high C/N

ratio litter and do not contribute in any substantial way to natural forest paedogenic processes. Beyond that, they are not well adapted to many forest environments. Most of the native legumes in BC do not behave as primary colonizers but rather are components of mature ecosystems. *Lupinus arcticus* may be the exception, but abundant growth of this lupine is limited to moister sites and it is rarely seen as an abundant primary colonizer except in those situations. Lupines also produce low C/N ratio litter (Domuta *et al.* 2007). Almost exactly the same thing could be said for alder, another nitrogen fixer that is ubiquitous, but only abundant in moister environments. The species which are typically considered as primary colonizers in BC forests such as lichens, bryophytes and legumes, can be found in abundance in a small minority of highly disturbed areas and are not currently found as the dominant primary colonizers on the vast areas of abandoned roads, gravel pits, cut banks, mines and other highly disturbed areas that can be found across BC.

We contend that the most common primary colonizer in forested British Columbia can be seen within a few minutes' drive from almost any town or city in the Interior of BC, but because we have not been conditioned to think of it as a primary colonizer, we don't see it as such. Across British Columbia from border to border and from the limits of the forest /grassland interface to the forest /alpine interface, lodgepole pine can be found growing in gravel pits, road cuts on landings and in highly disturbed areas of all kinds. To the chagrin of some, lodgepole pines readiness to establish has resulted in it being the most widely planted commercial tree species in BC igniting a controversy about too much pine plantation in BC. The most obvious evidence of the primary colonizer role of lodgepole pine is that in highly disturbed forest land across BC, it can be seen filling that role. Lodgepole pine has long been recognized as an early seral species, but now there is very good evidence to suggest why it can also behave as a primary colonizer.

The root architecture of naturally regenerating pine is well adapted to colonizing sites where soil compaction and limited water availability are problems. Though several reasons for this may exist, root anomalies in nursery stock have been well studied. The anomalous root architecture of nursery grown pine is likely a factor in the poor performance of planted pine in mine reclamation trials that have focused on planting nursery stock. Poor root form is likely more significantly detrimental in the harsher environmental conditions found in mine spoils. Limited research in BC has been conducted on planting pine seed in reforestation and reclamation. Early seeding trials focused on broadcast aerial seeding that resulted in high application rates and uneven seed distribution. Failures were anecdotally attributed to seed predation and germinant desiccation.

The Long Term Soil Productivity Trial is a network of trials across North America which is designed to look at the effects of soil compaction and organic matter removal on forest productivity. There are five installations with three replicates each in assorted biogeoclimatic zones across British Columbia. One of the more startling findings to come out of this network of trials is that the growth of lodgepole pine is not affected by various levels of removal of organic matter, including scalping the entire forest floor, nor is it affected by the most severe soil compactions (Kranabetter *et al.*, 2006). Even more extreme than this is the finding of Chapman and Paul (2012) that the growth and nitrogen levels of pine are not affected by growing on bare gravel as opposed to growing on adjacent intact soil. In addition Paul *et al.* (2009) have shown that nitrogen fixation occurs in tuberculate mycorrhizae on lodgepole pine at rates approaching

those found in alder. Pine has been used extensively to successfully rehabilitate hundreds of landings in British Columbia where the soil horizons have been scraped away. In short, lodgepole pine has all of the characteristics of primary colonizer for a wide range of forested biogeoclimatic conditions found in BC and it can readily be found filling that roll.

Because lodgepole pine utilizes internal nitrogen cycling relatively early in its lifecycle, its litterfall is not enriched in nitrogen as is the case for other nitrogen fixing species and so unlike, for example, alder litter, pine litter persists and forms forest floors which are the repositories of much of the nutrient capital in forest environments. An equally important attribute of lodgepole pine for reclamation is its ability to grow in compact soil. Parent material and highly disturbed soil all becomes compact before vegetation can establish because the roots, hyphae and other organisms that maintain soil structure in forest soils, are not present until after vegetation is well establish and cultivated soil quickly recompacts after rain or melting snow. The mechanisms which enable pine to grow in compact conditions are not well understood. Very sandy or gravelly soils do not have the same compaction limitations as finer textured soils but they present other challenges like poor nutrient and moisture holding capacity. Lodgepole pine can also have mycorrhizal symbionts on its roots that cause indeterminate, grass like root grow (Bravi, work in progress), which may be an adaptation coarse textured soils. Having site adapted mycorrhizal symbionts is likely one of the keys to lodgepole pines success on sites with so many different kinds of limitations.

The primary purpose of the work described here is to test whether or not modified direct seeding of lodgepole pine can be used to establish it as the primary colonizer on highly disturbed parent materials associated with mining. A secondary objective is to conduct a preliminary determination of seedling inoculation with microbial symbionts, in particular *Suillus tomentosus*. The primary challenges of this work are testing and developing delivery mechanisms for both seed and microbes. Seed delivery technology has been adapted to agricultural systems and it is unclear at this point how these technologies can be adapted for reclamation purposes with pine seed. Microbe delivery must be developed to ensure viability at the time at which germinants are receptivity to colonization, which could be a considerable time after germination.

METHODS AND MATERIALS

The research site is located at the # 3 Rock Dump on the Gibraltar Mine site property located near McLeese Lake, BC. The study site is approximately 3 hectares and is predominantly flat. About 1 hectare of the area has a north facing slope with a gradient of about 20%. Some minor material variability occurs throughout the study area. The waste rock is covered with approximately one meter of mostly medium textured overburden with pockets of higher clay soil (Table1). High winds are typical at the site.

Table 1: Soil Texture Analyses

Sample	% Sand	% Silt	% Clay
Gibraltar Free Dump Clay Vein	Hand texturing of this material indicated higher clay content than the other samples		
Gibraltar Free Dump Overburden	32.5	35	32.5
Gibraltar Overburden Pile Clay	37.5	32.5	30
Gibraltar Overburden Pile Dark	37.5	32.5	30
Gibraltar Overburden Pile Orange	55	22.5	22.5

SOIL TESTING

The study area was stratified into five similar types and 3 to 5 samples were taken from each stratum and composited. The samples were tested at the Provincial Research Soils Lab in Victoria for pH, total C, N, S, and CEC and exchangeable Al, Ca, Fe, K, Mg, Mn, Na, closed Vessel Microwave Digestion (HNO₃.HCl) ICP for Al, Ca, Fe, K, Mg, Mn, Na, and oxalate extractable Fe, Al and Mn.

BIOLOGICAL MATERIAL COLLECTION AND LABORATORY PROCEDURES

Local pine seed sources were identified with the assistance of Steve O’Hara and a cone collection was completed in May of 2011 from the lower tailings area on the Gibraltar property. A volume of 2.76 hl of *Pinus contorta* cones was collected over a 5 day period. Cones were placed in burlap bags and shipped to Silva Enterprises in Silva Enterprises in Prince George, BC where the seed was extracted. Seed quality was laboratory tested by placing seed on H₂O agar and determining germination rates. Germination rates for both stratified and unstratified seed was between 99 and 100%. Seed was stored at 4⁰C in a dry location to prevent mold formation (as per MOFR storage suggestions: <http://www.for.gov.bc.ca/hti/treeseedcentre/tsc/im1.htm>).

Suillus tomentosus was collected from pine stands in the same general area as the Gibraltar mine site.

Fresh sporocarps were brought to the lab where spore tissue was removed and dried in a small cabinet desiccator with gel desiccant or blenderized in dH2O and frozen. Live mycelia cultures were grown on nutrient agar from fungal cap tissue. Spore densities in suspensions were determined by conducting haemocytometer counts. Spore and live culture inocula were tested for viability on live seedlings growing on sterile medium in a growth chamber (Photo 1).



Photo 1: System used to observe mycorrhizal formation on germinants

Bacterial isolations were made from within *S. Tomentosus* tuberculate mycorrhizae collected from lodgepole pine stands, cultured on N-free medium to select for potential N fixing bacteria, and used in the inoculation trial. Bacteria from within the tubercles are currently being identified by DNA sequencing.

FIELD ESTABLISHMENT

Table 1 provides a summary of the treatments established. Bare seed planting was the predominant treatment type with approximately 2.5 ha's seeded across the area. Three mini-plots were established within the 3 ha area. Plots were sectioned into quarters and planted by hand with the microbial treatments.

Table 2. Treatment Summary - Gibraltar Mine Trial Establishment Paper			
Planting Date	Treatment	Seed Type	Planting
Fall 2011	Bare Seed	Unstratified	Hand seeded using PVC planting stick
Fall 2011	Bare Seed - Straw Matt	Unstratified	Hand seeded using PVC planting stick
Fall 2011	Soil Packet	Unstratified	Hand planted in plots
Fall 2012	Soil Packet - Straw Matt	Unstratified	Hand planted in plots
Fall 2013	Microbe Pellet	Unstratified	Hand planted in plots
Fall 2014	Microbe Pellet - Straw Matt	Unstratified	Hand planted in plots
Fall 2015	Live Microbe Pellet	Unstratified	Hand planted in plots
Fall 2016	Live Microbe Pellet - Straw Matt	Unstratified	Hand planted in plots
Fall 2017	Capsule	Unstratified	Hand planted in plots
Fall 2018	Capsule - Straw Matt	Unstratified	Hand planted in plots
Spring 2012	Bare Seed	Stratified	Hand seeded with grass seeder

BARE SEED PLANTING

Three hand seeding implements were tested, the Johnny Seedstick (Photo 2), a PVC pipe planting stick

(Photo 3) and the Earthway Ev-N-Spred Seeder. The Seedstick needed to be modified to withstand the rocky planting conditions and in the end the PVC pipe was used to conduct the fall plant and the Earthway Ev-N-Spred seeder was used to complete the spring seeding.



Photo 2 and 3: Johnny Seed Stick and PVC pipe planting implements

MICROBE DELIVERY AND SEEDING

Four approaches were used for microbe delivery including spores entrained in a medium designed to provide protection from spore predation and desiccation, live mycelium in an enteric capsule that was designed to disintegrate close to seed germination, live mycelium in a medium designed to supply nutrient for growth and protection against predation and desiccation and soil inoculation with soil taken from a vigorous pine stand in same geographic region as the mine and with some of the same establishment challenges present in the mine site.

Approximately 100 g packets of soil inoculum were created for ease of handling in the field. Pine seed was contained within each packet so that germinants would be in intimate contact with inoculum. Seeding with inoculation systems and bare seed treatments all took place in October 2011. A bare seed treatment was also applied in May 2012.

EROSION CONTROL

Erosion Control S31 Straw blanket from Erosion Control of Winnipeg, Manitoba was used on an approximately 0.5 ha portion of the sloped portion of the trial area to test its effectiveness at controlling erosion until vegetative cover is established and to determine if it interferes with germination success.

RESULTS AND DISCUSSION

Good candidate fungi for inoculation were collected and stored in various ways. All of the mycorrhizal inoculum delivery mechanisms proved capable of initiating infection in microcosm lab trials and *S. tomentosus* specific morphologies developed on lab inoculated seedling roots.

The PVC pipe is easy to use and fail proof but the delivery of the seed into the planting bed is imprecise and seed depth and placement are difficult to evaluate. The Ev-N-Spred seeder broadcast delivered seed at about 10,000 seeds per hectare with a good distribution. Most of the soil had a neutral to slightly basic pH but one small stratum had a pH of 4.4 and elevated exchangeable aluminum and manganese, both of which are mobilized under acid conditions. Both of these elements can be harmful to germinants and any localized germinant failures will need to be examined to determine if toxic elements are playing a role. The high clay content of the soil may pose a challenge if it breaks down structurally before germinants can establish.

Local seed collection proved to be economically feasible and resulted in very good quality seed. Direct seeding of the pine presented no major technical obstacles and would be feasible for mine reclamation with very simple technology. Seedbed preparation was not ideal. The soil was driven over repeatedly and was structurally broken down. The soil surface was too level as if the site had been prepared for planting grass. Surface roughness provides wind protection and traps moisture in micro sites, and would have been more conducive to lodgepole pine establishment.

The trial will be monitored at regular intervals in 2012 and into the future. One of the most important factors to monitor will be the degree and type of mycorrhization. We consider the formation of tuberculate mycorrhizae to be a critical measure of success. By fall 2012 we should have a good idea of whether or not success is likely.

REFERENCES

Barbour, Michael G.; Billings, William Dwight (2000). North American Terrestrial Vegetation. Cambridge University Press

Chapman, W.K., and Paul, L. R. 2012. Evidence that northern pioneering pines with tuberculate mycorrhizae are unaffected by varying soil nitrogen levels. Microb. Ecol. doi 10.1007/s00248 012-0076-0.

Domuța, C., Ciobanu, G., Șandor, M., Samuel, A., Ciobanu, C., Sabău, N., Șcheau, C.V., Borza J., Domuța, C. 2007. Green manure technology with c/n ratio favorable for humus accumulation and their influence on maize yield. Research Journal of Agricultural Science. Vol 39, No 2

Kranabetter, J. M., Sanborn, P. Chapman, B. K. and Dube, S. 2006. The Contrasting Response to

Soil Disturbance between Lodgepole Pine and Hybrid White Spruce in Subboreal Forests. *Soil Sci. Soc. Am. J.*, Vol. 70, September–October

D. J. Read. 1991. Mycorrhizas in ecosystems. *Cellular and Molecular Life Sciences*, 1991, Volume 47, Number 4, Pages 376-391

Schmidt, M.W.I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner S., Trumbore, S. E. 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478. Pp 49–56