Growing Functional Nitrogen-Fixing Native Trees and Shrubs for Land Reclamation in British Columbia

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Native actinorhizal trees and shrubs have the ability to fix atmospheric nitrogen and improve soil nitrogen concentrations. This is of particular value for the reclamation of low nutrient mine wastes. Nursery studies were conducted to assess practical methods for inoculating *Alnus viridis* ssp. *sinuata*, *Elaeagnus commutata*, and *Shepherdia canadensis* seedlings with appropriate strains of *Frankia* bacteria. Results indicated that application of a liquid inoculum achieved good success. An additional application method using Mikro-Beads™ is also discussed.

INTRODUCTION

Revegetation of mine soils is often limited by coarse-textured materials with high coarse fragment content and low nutrient status. Application of fertilizer, either as an inorganic commercial product or as an organic waste product, is typically used to initiate a nutrient pool in the mine soil. Legume species such as clover or alfalfa which have rhizobial associations that fix atmospheric nitrogen are often seeded to improve the nitrogen content of the soils. These plants have value as forage for range cattle and other animals, but at some mine sites proposed land uses such as forestry or wildlife habitat require the establishment of woody species. In these instances the use of actinorhizal perennial woody trees and shrubs can fix atmospheric nitrogen and increase the soil nitrogen content. In addition to improving the soil nitrogen levels, actinorhizal plants ameliorate soil temperature and moisture conditions through the accumulation of organic matter resulting from leaf and root litter deposition and decomposition (Visser et al., 1991).

Various species of actinorhizal shrubs are grown in western Canada for use in mine land reclamation. These species include *Alnus rubra* (red alder), *Alnus viridis* ssp. *sinuata* (Sitka alder), *Ceanothus velutinus* (snowbrush), *Elaeagnus commutata* (wolf-willow), and *Shepherdia canadensis* (buffaloberry). While these plants have the capability for association with a bacterium (*Frankia* spp.) and the potential to form root nodules, this often does not occur in container-grown nursery stock. A survey conducted of seven nurseries located in Alberta and British Columbia indicated that *E. commutata* and *S. canadensis* seedlings did not become nodulated in their first year and that planting stock generally lacked nitrogen-fixing ability (Danielson and Visser, 1990). The conclusion of this survey supported our observation that container-grown nursery stock of *E. commutata* and *S. canadensis* were lacking *Frankia* nodulation. In monitoring programs conducted at various mine sites planted with these two species it was also our observation that the initial growth of these two species planted on reclaimed mine sites was poor. The soils at these mine sites, in addition to having

low nutrient conditions, did not contain potential *Frankia* inoculum. Therefore, without a source of *Frankia* inoculum *Shepherdia* and *Elaeagnus* displayed similar growth to other non-actinorhizal, species planted on these sites. Occasionally, a number of years subsequent to planting, the actinorhizal species would begin to grow rapidly and could be shown to have become nodulated. This was particularly noticeable with *S. canadensis* where the leaf color would change to a dark green shade. This type of on-site nodulation must be due to inoculum from adjacent forest areas being transferred to the reclaimed site.

METHODS

It became apparent that for us to utilize these actinorhizal plants for their nitrogen-fixing abilities, it would be necessary to ensure that the seedlings were inoculated with the appropriate *Frankia* species before they were planted on the mine sites. We experimented with collecting nodules from plants growing in natural forest sites and applying a slurry of the ground nodules to our nursery stock. The results from these initial experiments had limited success. We then contacted Mikro-Tek, an Ontario company with experience in growing bacterial cultures of *Frankia* for the inoculation of *Alnus*. At that time they had not grown *Frankia* inoculum for either *Shepherdia* or *Elaeagnus*, but believed they could provide us with a suitable culture. We collected nodulated roots from *Shepherdia* and *Elaeagnus* and sent them in coolers to their lab where they processed the nodules and initiated the cultures. The growth of these *Frankia* species were much slower than Mikro-Tek had experienced with other *Frankia* cultures, but with adjustments to their media they were able to culture these bacteria. We also collected nodules from a northwestern *A. viridis* ssp. *sinuata* population and they also provided us with a suitable culture for this *Alnus* seed source.

We have applied *Frankia* to our seedlings using two methods: the bacterial culture can be directly watered onto the seedlings or it can be mixed into the soil media if the bacteria is encapsulated in peat moss beads called Mikro-Beads™. The first application technique is relatively simple, the inoculum can be hand watered onto the seedlings or can be introduced into the overhead watering system. To utilize an overhead watering system, it is important to remove any filters in the water system which could trap the bacteria. In the second application technique, it is important to mix the appropriate number of Mikro-Beads™ into the soil media to ensure that the bacteria are evenly distributed to each cavity and available to the young roots. The advantages of the Mikro-Beads™ method is the ability to store the product for a longer period over a wider range of temperature conditions. The liquid cultures are shipped in a growth media and must be shipped and stored in refrigerated conditions. The liquid cultures must be applied quickly after reaching the nursery; therefore, it is important to be able to predict when the seedlings will be available for inoculating. The *Frankia* bacteria infect the young growing roots and it is necessary to have the seedlings at the right stage of development when inoculating with the liquid culture. The timing is not as critical for the Mikro-Beads™ which can be incorporated into the top 2 or 3 cm of the soil media at the time of planting.

Most of our seedlings of *A. viridis* ssp. *sinuata*, *E. commutata*, and *S. canadensis* were inoculated in the spring of 1996 using the liquid inoculum method. Two hundred of each species were maintained as a control which did not receive

inoculum. In 1997 most of the treated seedlings were treated with the Mikro-Beads™, but some were treated with the liquid inoculum and two hundred were maintained as an untreated control.

RESULTS

We assessed the results of the application of the *Frankia* inoculum on *S. canadensis*, *E. commutata*, and *A. viridis* ssp. *sinuata* in September 1996, approximately 5 months after the date of application. Samples were selected randomly from these populations and visually inspected. Of the *Frankia*-treated *Alnus* stock, 75% had nodules which were easily visible; however, 68% of the untreated control *Alnus* seedlings also had visible nodules. Since our nursery is located adjacent to a forest containing *A. rubra* it is likely that spores of *Frankia* naturally inoculate much of our *Alnus* stock. Nodules were not visible on any of the *Elaeagnus* or *Shepherdia* seedlings. A subsample of *Shepherdia* seedlings were shipped to Mikro-Tek where they were assessed. They gently washed the root systems to remove the soil and then observed the roots under a microscope at 20× magnification. All of the root systems were observed to have nodules and ranged from 4 to 10 nodules per plant. Most of the nodules were small, less than 2 mm in diameter, and only one had multiple lobes. All of the nodules were located at the bottom of the growing plug near the drainage opening.

DISCUSSION

The results from the inoculation of the 1997 crops are not yet available, but it is hoped that the use of the Mikro-Beads™ will be as effective in the establishment of *Frankia* populations as was the liquid inoculum used in 1996. While the liquid inoculum is easily applied, the shipping and storage of the material is limited by both temperature and time. Use of the Mikro-Beads™ is more practical for nursery management.

In a field trial conducted on oil sands tailings, Visser et. al. (1991) reported that both *Elaeagnus* and *Shepherdia* had greater height growth, and produced heavier shoots and roots when inoculated with soil containing *Frankia* than did the uninoculated controls. We intend to monitor the growth response of the actinorhizal shrubs grown in our nursery that have been planted in various mine reclamation projects. We expect that the successful inoculation of these species with appropriate *Frankia* species in the nursery will result in superior growth at the mine sites.

LITERATURE CITED

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