THE ROLE OF SYMBIOTIC MICROORGANISMS IN REVEGETATION OF DISTURBED AREAS - REDWOOD NATIONAL PARK

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Abstract. Symbiotic microorganisms play an important role in initial revegetation of disturbed areas. Nitrogen fixation and mycorrhizal associations improve the ability of plants to grow in and enrich nutrient poor soils. Establishment of these relationships on nursery stock prior to outplanting improves outplanting survival. Implementation of large scale inoculation resulted in increased survival and initial growth rates. In a forest nursery *Thelephora terrestris* survived sterilization processes for sytroblocks and provided inoculum for the following years crop of Douglas fir. Preliminary inoculations of redwoods with *Glomus fasiculatus* has achieved some mycorrhizal formation.

INTRODUCTION

The role of soil microorganisms in the ecosystem has long been poorly derstood when compared with the knowledge of macroscopic organisms. However, th increased efforts to establish vegetation on disturbed sites, the vital ture of the roles of these groups is gaining greater recognition. Symbiotic il microorganisms increase the availability, accumulation and cycling ficiency of many essential plant nutrients. These microorganisms improve e ability of plants to grow on nutrient deficient soils, and are essential the success of many higher plant species in their natural habitats.

Soil erosion and nutrient loss caused by disturbances to mature forest osystems can be reduced by the rapid revegetation of disturbed sites. tablishment of vegetation on recently disturbed bare soil is often complited by harsh site conditions and lack of plant available nutrients. Nitrogen xing plant species are often important components of early succession creasing the absolute amounts and availability of soil nitrogen and soil ganic matter content (Tarrant & Miller 1963, Youngberg & Wollum 1968). rbon to nitrogen ratios of above and below ground litter components are rrowed by nitrogen fixing species (Cromack & McNabb 1979).

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The below ground component of forest ecosystems is an important current area of research in nutrient cycling and decomposition. In many forests, most energy utilization and nitrogen transformations of carbon components occur below ground (Harris et al. 1980; Fogel 1980). This is true throughout secondary succession. Above ground patterns of changes in species composition and vegetative structure with forest succession are accompanied by major qualitative changes below ground. One stage of succession is affected by prior stages that occur below ground. Symbiotic soil microorganisms play a key role in the formation of these below ground ecosystems.

Two types of organisms fix atmospheric nitrogen to a plant available form in association with vascular plant roots. Bacteria of the genus Rhizobium form nodules in relationships with legumes. A group of woody shrubs and trees form root nodules with Actinomycetes. One such associate is red alder, annually fixing as much as 320 kilograms per hectare in the coast rages of Oregon (Newton et al. 1968). These relationships provide large quantities of nitrogen the nutrient most commonly limiting in disturbed forest soils.

Mycorrhizal relationships of two basic types occur on most species of vascular plants. Ectomycorrhizae form between fungi and the roots of most conifers and broad-leafed trees as well as many woody shrubs. Endomycorrhizae are relationships between fungi and most herbaceous plants, some conifers and woody broad-leafed plants. Both associations aid the vascular plant symbiont by increasing effective surface area for absorption, increasing ability to take up P, N, and cations (Melin et al. 1950, 1952, 1958). Resistance to some serious diseases is increased by the production of antibiotics (Marx and Davey 1968). Fungi obtain habitat and carbohydrates from their photosynthetic symbionts (Melin and Nilsson 1957). The specific characteristics of mycorrhizal associations vary with the species forming them.

In Redwood National Park (Table A) and the western states (Klemmédson 1979, Rose 1980), many important woody plants invading freshly disturbed sites are nitrogen fixing as well as mycorrhizal. Nitrogen fixation enables rapid growth due to increased nitrogen availability. Mycorrhizae increase the uptake of other nutrients, reducing their tendency to become limiting with increased growth due to nitrogen availability. Plants forming both these relationships can thrive in conditions of low soil nutrient levels. With attempts to reestablish vegetation on disturbed sites, the importance and potential benefits of these relationships are being recognized. Utilization of these organisms holds great potential and is an integral part of any complete natural vegetation system restoration.

The Redwood National Park watershed rehabilitation program includes revegetation of areas immediately following heavy equipment work. These sites contain the most highly disturbed vegetation systems in the Redwood Creek basin. Soils consist largely of loose, subsoil fill or ripped road surfaces, often deficient in plant available nutrients. Decomposition of mulches applied to minimize surface erosion initially reduces plant available nitrogen. Recently excavated soils are typically deficient in microbial flora, reducing potential for rapid formation of mycorrhizal and nitrogen fixing relationships (Wilson 1965). Proper establishment of microflora on planting stock can have significant impact on the success of this or any large scale revegetation effort.

TABLE A. SYMBIOTIC ASSOCIATIONS OF PLANT SPECIES USED IN THE REDWOOD NATIONAL PARK REVEGETATION PROGRAM

Species	Nitrogen Fixing		Mycorrhizal	
	actinomycete	Rhizobium	ecto-	endo-
Acer macrophyllum	0	0	+[1]	+[1]
Alnus oregana	+	0	+[2]	+[3]
Arbutus menziesii	0	0	+[4]	0
Baccharis pilularis var. *consanguinea*	0	0	0	+[1]
Carex spp.	0	0	0	+[1]
Ceanothus thrysiflorus	+	0	0	+[3]
Corylus cornuta var. *californica*	0	0	+[5]	0
Gaultheria shallon	0	0	+[4]	+[4]
Grasses	0	0	0	+[1]
Juncus spp.	0	0	0	+[1]
Legumes	0	+	0	+[1]
Lithocarpus densiflora	0	0	+[5]	+[1]
Pseudotsuga menziesii	0	0	+[5]	0
Rhododendron macrophyllum	0	0	+[4]	0
Rubus parviflorus	0	0	0	+[1]
Rubus spectabilis	0	0	0	+[1]
Rubus vitifolius	0	0	0	+[1]
Salix spp.	0	0	+[5]	+[1]
Sambucus callicarpa	0	0	0	+[1]
Sequoia sempervirens	0	0	0	+[1]
Vaccinium ovatum	0	0	+[4]	+[4]
Whipplea modesta	0	0	0	+[1]

[1] Data obtained from material collected in Redwood National Park.

[2] Molina, 1980.

[3] Rose, 1980.

[4] Largent, *et al.*, 1980.

[5] Trappe, 1962.

To determine the status of commercially available planting stock, a survey of symbiotic relationships was conducted (Table B). Roots of seedlings were stained with trypan blue and examined for endomycorrhizae. Other rootlets were sectioned and examined for the Hartig net and mantle which are diagnostic of ectomycorrhizae. Root systems of plant species known to form associations with nitrogen fixing organisms were examined visually for evidence of root nodules.

TABLE B. SYMBIOTIC RELATIONSHIPS PRESENT ON NURSERY STOCKS USED BY REDWOOD NATIONAL PARK

Plant Species	Age	Type	Associations	Percent Infection	Symbiont
Redwood	2.0	BR*	endomycorrhizal	100%	unidentified
Redwood	1.0	SB**	none	0%	---
Redwood	1.0	PT***	none	0%	---
Douglas-fir	2.0	BR	ectomycorrhizal	99%	Laccaria lacata and Telephora terrestris
Douglas-fir	1.0	SB	ectomycorrhizal	95%	T. terrestris
Douglas-fir	1.0	PT	none	0%	---
Red alder	1.0	PT	none	0%	---

* BR: Bare Root Seedlings; outdoor field grown.

** SB: Containerized seedlings in styroblocks; greenhouse grown.

*** PT: Containerized seedlings grown in plastic tubes; greenhouse grown.

MYCORRHIZAL ASSOCIATIONS

Virtually all of the bare root seedlings had formed extensive mycorrhizal associations. L. lacata and T. terrestris were present in large quantities in association with Douglas-fir. Both of these species are common to nursery beds.

Redwood seedlings in nursery beds were heavily infected with endomycorrhizal fungi. The identification of these fungi is pending spore production. All bare root seedlings were grown outdoors in plowed, fumigated fields.

Douglas-fir seedlings grown in styrofoam blocks (styroblocks) were the only containerized seedlings found to be forming any mycorrhizal associations. Seedlings grown in plastic tubes under very similar conditions were devoid of ectomycorrhizae. T. terrestris was the fungal symbiont of all 95 percent of the Douglas-fir found to be ectomycorrhizal. All containerized seedlings were grown in greenhouses or under shade frames.

:tomycorrhizae

Controlled greenhouse studies investigated the ability of T. terrestris) survive in and around styroblocks. Douglas-fir seedlings were grown in new ıd used blocks placed directly on the surface of a wooden table. Containers :re isolated from each other to prevent spread of infection. An average of .3 percent of the seedlings were ectomycorrhizal in new blocks while 82.8 :rcent of the seedlings in recycled styroblocks formed mycorrhizae with T. :rrestris. The large difference in the rate of infection suggests that the urce of inoculum is related to the recycling of the containers. This was ıt unexpected due to the presence of abundant T. terrestris mycelia and uiting bodies on the surface of the containers. To function as a source inoculum, fungal hyphae would be required to survive the washes of water d dilute bleach solutions intended to sterilize the blocks.

To determine the ability of the T. terrestris to spread between styro-ocks another groups of seedlings was grown. New and recycled blocks were aced adjacent to each other in an alternate pattern on the table. Another t of new blocks were placed adjacent to the previously used ones but evated to prevent direct contact with the table. Rate of infection in new yroblocks placed adjacent to recycled was greatly increased over the rate isolation. Elevated containers had the lowest infection rate of any eatment (3.4%). Observation of the underside of the containers revealed celia growing out of the bottoms of containers, spreading to other chambers d adjacent blocks in the space between the containers and the table. In is manner the mycelium spread rapidly through the nursery and provided oculum for the following year.

lomycorrhizae

Coast Redwood, unlike most conifers, forms endomycorrhizae and not :omycorrhizae. Available field grown bare root seedlings had uniformly ıvy infections. Containerized seedlings grown in greenhouses or under ıde frames were all sterile (Table B).

Little information is available on the endomycorrhizae formed by redwood. oculations performed on one year old redwood seedlings, with Glomus :iculatus, produced some infections but no growth response. Endomycorrhizae med displayed good vesicles and arbuscles but did not become widespread on root systems. This study is being conducted under greenhouse conditions ng containerized redwood stock.

NITROGEN FIXING ASSOCIATIONS

zobium Nodulated

Legumes are commonly utilized for their ability to fix nitrogen. Commer-l inoculum is available for many species. Legumes were inoculated and ded on rock subsoils producing dense stands without fertilization. The lity to grow rapidly and add nitrogen and organic matter to the soil makes se plants valuable in restoring disturbed areas.

Actinomycete Nodulated

The revegetation program in Redwood National Park emphasizes the establishment of successional plants as well as potential canopy species. On moister slopes red alder is the most effective native woody plant colonizing bare soil, rapidly forming dense stands over large areas following any ground disturbance. C. thrysiflorus is adapted to colonization of sites following fire, forming dense stands following slash burning (Muldavin et al., 1981). Due the their ability to form associations with nitrogen fixing actinomycetes these species are important in soil restoration and nutrient enrichment. For this reason red alder is being used as a primary species where the rapid establishment of vegetation on a moist slope is required.

For slope stabilization it is important to maximize initial survival and growth rates. Large scale red alder plantings warranted investigation of the effects of nitrogen fixing actinomycetes on survival and initial growth after outplanting. No evidence of nodule formation was found on the seedlings available for purchase. After one season of growth unnoculated, outplanted seedlings averaged 93 percent increase in top height. A test of nodulated seedlings averaged 526 percent increase. Initial height and site conditions were similar for the two samples. Following the first growing season after outplanting, all seedlings were nodulated. Size advantage gained from the increased initial growth, enables greater initial growth and vigor the following year.

The 1980 revegetation program included the development and implementation of inoculation procedures for actinomycetes on containerized alder seedlings. Sterile one year old seedlings were transplanted into glass sided containers to observe root development and nodulation. Half of the seedlings were inoculated with an aqueous suspention of ground nodules. Dosage was 0.50 grams of nodule per container. Seedlings were grown under greenhouse conditions. Root and shoot growth were carefully monitored.

Twenty-eight days after inoculation, all treated seedlings showed signs of nodulation while the controls did not. Fourteen days later, leaves begun to rapidly increase their size and darken in color. A rapid increase in growth rate followed immediately. Control plants gradully become chlorotic, leaf size decreased and no top growth was observed. During the first growing season following inoculation, caliper and top height were increased by 325 percent and 1257 percent respectively, over the control plants.

Practical use of this technique for large scale inoculations of nursery stock requires more precise knowledge of the minimum inoculation dosages required to achieve a high rate of nodulation. Seedlings were inoculated at rates ranging from 0.001 to 0.250 grams nodule per container and grown under greenhouse conditions. At 0.080 grams per container and above, 100 percent nodulation was achieved. Gradual reduction occurred form 0.003 to 9 percent at 0.001 grams per container. Inoculation at the rate of 0.003 grams per container produced higher rates of nodulation when seed, rather than one month old seedlings, were inoculated.

Fifty thousand containerized red alder seedlings were inoculated one month after seed was sewn for the 1980 planting season. Inoculum was prepared by masceration of field collected nodules in a blender at high speed in sterile water. The solution was strained and diluted to a concentration of

0.080 grams nodule per mililiter to simplify inoculation procedures. Treatment was at a dosage rate of 0.080 per gram, or one mililiter per container. Inoculations were performed by spray misting 10,000 seedlings and with the use of a repeating pipetter for the remaining 40,000. Seedlings were otherwise grown under normal nursery conditions until outplanting.

Prior to the dropping of the leaves, the inoculated areas of the greenhouse were visually distinct from the uninoculated areas. Nodulated plants appeared taller with darker, larger leaves and were readily distinguished from unnodulated plants. Distinct lines were apparent between the inoculated and uninoculated groups of plants adjacent to each other. After plants were dormant, random samples were removed and root systems examined for the presence of nodules. The abundance and relative stage of nodule development displayed by the individual seedlings was noted. Nodulation rate varied from 60-95 percent in different samples with no difference observed between the two methods of inoculation. This rate of nodulation is appreciably reduced from the small scale preliminary tests. This is attributed to reduced precision and accuracy of the large scale inoculations and to application of fertilizers and pesticides used in commercial nurserys. These factors were not included in the preliminary tests.

At the time of outplanting the size and development of seedlings was improved. Nodulated seedlings averaged 20.3 percent increase in top height and 16 percent increase in caliper over the unnodulated trees. The seedlings with well developed nodules had 32 percent and 41.7 percent increases respectively, over the unnodulated. The overall vigor of seedlings was improved.

An outplanting test plot was established to monitor the effects of nodulation after outplanting. Nodulated and unnodulated seedlings were planted in alternate rows. Initial growth for the nodulated trees was rapid with lush green foliage, large leaves and green stems. The uninoculated seedlings uniformly displayed slower growth much smaller chlorotic leaves and red stems. After twelve weeks all trees appeared healthy, dark green and robustly growing. Six months after planting, top heights were measured. An average increase of 63.7 percent was obtained for nodulated seedlings over unnodulated. The inoculations appeared to have reduced planting shock and seedlings appeared much more robust with more and stouter lateral branches. Mortality rate was lower in the nodulated sample and recovery from wildlife browsing was much more rapid.

DISCUSSION

Establishment of vegetation on recently disturbed bare soil is made difficult by the lack of plant available nutrients. Without nitrogen fixing and mycorrhizal symbionts, growth and survival rates are poor for planted seedlings, even for species well adapted to planting sites. The obvious increase in vigor, due to establishment of nodules on nursery grown red alder, shows high potential in establishment of these relationships on seedlings. Rate of nitrogen fixation and growth response are known to be even higher, with the additional presence of mycorrhizae. More effective use of nitrogen fixing species for slope stabilization, and as a nitrogen source in reforestation, can be achieved with establishment of symbiotic relationships prior to outplanting.

Establishing mycorrhizal associations on nursery seedlings increases survival, growth and resistance to pathogens. However, work with T. terrestris exemplifies the complex nature of establishing mycorrhizae on nursery stock. Though abundant on nursery stock, it is not always easily established or controlled if another species is determined more desirable. The ability of this fungus to produce antibiotics is well documented, but planting tests confirmed those of Marx (1975) which obtained no effect on initial survival rates. Survival and vigor at another stage may be influenced. To achieve maximum results, techniques must utilize a wide range of mycorrhizal species. The information concerning growth and spread of T. terrestris in nursery situations, will probably be most applicable to the establishment of other species.

The utilization of symbiotic soil microorganisms in revegetation of disturbed areas holds great promise. Naturally occuring vegetation is reliant on microorganisms for nutrient availability and rapid growth. We must also rely on these relationships. Without them, many invading species would not approach the success they display in colonizing disturbed sites.

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