THE ROLE OF SYMBIOTIC MICRO-ORGANISMS IN POST-DISTURBANCE ECOSYSTEMS, REDWOOD NATIONAL PARK

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ABSTRACT

Plant species dominating undisturbed vegetation systems are dependent on mycorrhizal associations for optimal nutrient uptake and cycling. Following disturbances of logging, fire and erosion, plants which form both nitrogen-fixing and mycorrhizal symbioses become dominant. Advantages gained by these relationships enable rapid growth during colonization. Nursery establishment of nitrogen-fixing organisms with *Alnus oregona* seedlings increased initial planting stock size and vigor as well as survival and growth upon outplanting. Inoculated clover rapidly became dominant on recently recontoured roads. Conifer seedlings exhibited higher growth and survival rates when appropriate mycorrhizae were established in the nursery. Understanding the roles of symbiotic micro-organisms enables their utilization in management of ecosystem recovery and maintenance.

INTRODUCTION

The below ground component of ecosystems is currently an important area of research in nutrient cycling and decomposition. Symbiotic soil micro-organisms play an important role in cycling nutrients of undisturbed vegetation systems and in initial revegetation of disturbed systems. Nitrogen-fixing and mycorrhizal associations increase availability, accumulation and cycling efficiency of many essential plant nutrients. Both mycorrhizae and some nitrogen fixing plants also produce antibiotics which inhibit the growth and spread of important forest pathogens (Marx and Davey 1968). Improved growth enables competitive advantage on nutrient poor soils, and is essential to the success of many higher plant species in their natural habitats.

Mycorrhizal associations are present under natural conditions on almost all species of higher plants. These associations increase the rate of P, N, and cation uptake by the plant while providing carbohydrates to the fungal symbiont (Melin and Nilson 1950, 1952, Melin and Hakskaylo 1958). Fungi aid in uptake and increase litter decomposition rates, maximizing the efficiency of nutrient cycling and establishment of stable systems.

Certain bacteria and actinomycetes inhabit nodules in association with compatible dichotoledonous plants to form symbioses capable of fixing atmospheric nitrogen to a plant usable form. These nodules are formed with plants which are also forming mycorrhizae. Large quantities of nutrients available allow these plants to grow extremely rapidly.

In absence of disturbance, vegetation communities in Redwood National Park are dominated by plants solely dependent on mycorrhizal associations for nutrient cycling and uptake. Species which form both nitrogen fixing associations and mycorrhizae remain at low densities and do not comprise a major part of the canopy or understory vegetation.

Species with rapid initial growth rates are often most successful in colonization of areas after disturbance has removed vegetative cover. Soil erosion and nutrient loss caused by disturbances to mature ecosystems can be reduced by rapid revegetation. Nitrogen fixing plant species are important components of early succession (Klemmedson 1979), increasing the absolute amounts and availability of soil nitrogen and organic matter content (Tarrant and Miller 1963, Youngberg and Wollum 1976). One such associate is *Alnus oregona,* reported to fix as much as 320 kg/ha annually in the coast ranges of Oregon (Newton et al. 1968). Biological nitrogen fixation provides large quantities of the nutrient most commonly limiting in disturbed forest soils.

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Both natural and man-caused disturbances have had significant impact on vegetation systems covering most of the 48,000 ac 1978 expansion of Redwood National Park. Approximately 35,000 ac of old-growth stands dominated by coast redwood *Sequoia sempervirens* and Douglas-fir *Pseudotsuga Menziesii* were cut prior to acquisition. Approximately 2,000 ac of prairie and 600 ac of oak woodlands have been grazed since the arrival of European man in the 1850s. The area has steep slopes with high natural erosional disturbance greatly increased by recent logging. Fire has historically occurred by lightning strikes and probably periodic burning by Native Americans. In recent years, fire has been used as a post-logging treatment to prepare the site for conifer regeneration and natural fires have been suppressed.

Nitrogen fixing plant species do not dominate any undisturbed systems in Redwood National Park, but are commonly dominant as post-disturbance colonizers. Four major types of vegetation have rapidly colonized disturbed areas and are presently dominated by species which are both mycorrhizal and nitrogen fixing. Moist mesic clearcuts support dense, rapidly growing *A. oregona* stands. Dry mesic to xeric clearcuts which were burned are occupied by dense *Ceanothus thyrsiflorus* chaparral. Prairie gullies, active slides, and oak woodlands after burning are colonized by heavy lupine stands. Prairie sites with heavily disturbed soil surfaces are colonized by dense mats of clovers. Each of these dominants rapidly colonizes bare soil, but is eventually outcompeted in absence of continued disturbance.

With re-establishment of stable, non-disturbance related systems, nitrogen fixing plant species do not maintain dominance. Plant species of the mature systems benefit from colonizers by improved soil conditions, reduced erosion, and the reduction of pathogens. Mycorrhizal associations maintain efficient nutrient cycling and disease resistance in mature ecosystems.

The purpose of this study was to evaluate the role of symbiotic micro-organisms associated with important plant species in post-disturbance ecosystems in Redwood National Park.

METHODS

Utilization of symbiotic micro-organisms in ecosystem recovery requires differentiation between methods establishing colonizing nitrogen fixers and the long-term system recovery dependent on mycorrhizae. Establishment of nitrogen fixation on outplanted and seeded plants increases initial growth and effectiveness in colonizing after disturbance (Sugihara and Cromack 1981). The presence of proper mycorrhizae on longer term revegetation plantings increases survival and growth, reducing the time required to overtop colonizers (Fogel 1980). The following studies were conducted with plants used in the revegetation program.

Ectomycorrhizae

A survey of Douglas-fir ectomycorrhizal associations present on locally available conifer seedlings revealed virtually all bare root seedlings had abundant infections. Seedlings grown in containers had no mycorrhizae with the exception of those grown in recycled styroblocks.

All infections were by *Thelephora terrestris* which is known to produce beneficial antibiotics and provide ozone protection to the host tree roots. To determine if the source of inoculum was the recycled blocks, seedlings were greenhouse grown in isolated new and recycled blocks. New and recycled blocks were also arranged on the table to determine if the fungus has the ability to spread, providing an inoculum source.

Endomycorrhizae

Bare root coast redwood seedlings grown outdoors in plowed fields all formed abundant endomycorrhizae. Greenhouse, container grown seedlings had none. To determine the ability to form mycorrhizae with *Glomus fasiculatus,* sterile seedlings were grown in the greenhouse. At the age of 1 yr, spores of *G. fasiculatus* were placed on young rootlets. Control and inoculated plants were grown another year, roots were removed and stained for endomycorrhizae.

Legumes

Legumes are commonly utilized for their ability to fix nitrogen. Seed as well as *Rhizobium* bacteria inoculum are commercially available. In experimental seeding, *Trifolium subterraneum* was seeded on recently filled terrain both in prairies and forest situations. Soil was new fill from rehabilitated road beds. Seed was inoculated with a commercial mix and spread at 16.9 kg/ha with 39.1 kg/ha grass mix. Cover for clover was measured the first and second years following treatment (Reed and Hektner 1983).

Actinomycete Nodulated Nitrogen Fixers

(a) Alder nursery stock purchased for outplanting in erosion sensitive areas had not formed any nitrogen fixing associates. Unnodulated seedlings were measured and outplanted in comparison with field collected nodulated seedlings. After one growing season heights were remeasured.

(b) To test the feasibility of artificial inoculation of nursery stock, an aqueous suspension of ground nodules was made by blending at high speed with sterile distilled water. This solution was placed in glass sided containers with sterile alder seedlings. Control plants were treated in the same way without inoculation. Seedlings were greenhouse grown, watered with sterile water and observed for signs of nodulation and nitrogen fixation for 1 yr.

(c) Determination of dosage required for nodulation was tested by inoculating at 0.001 to 0.250 g nodule/tree. Seedlings were kept for one growing season, then roots were washed and checked for nodulation.

(d) For the 1980 planting season alder seedlings were inoculated at a rate of 0.080 g nodule/seedling. At the age of one month, 10,000 seedlings were spray misted and 40,000 were inoculated with a repeating pipetter. Other growing conditions were not varied from standard commercial procedures. Prior to outplanting, samples of both inoculated and uninoculated seedlings were observed to assess results.

(e) To evaluate the effects of induced nodulation on growth after outplanting, a test plot was set up. Trees were tagged and planted in alternating rows of nodulated and unnodulated. Seedlings were measured initially and at one and 2 yrs to determine relative survival and growth rate.

RESULTS AND DISCUSSION

Ectomycorrhizae

Douglas-fir seedlings grown in previously used (recycled) styroblocks had nearly 20 times the rate of infection of new blocks. Both types were grown in isolation with no opportunity for mycelial spread between blocks. When grown adjacent to recycled containers, new styroblocks had comparable infection rates (56.4 - 71.5%) to the recycled. However, when elevated to prevent mycelial spread on the stable surface, infection rates fell to 3.4%.

Table 1

Infection Rates for Douglas-Fir Treatments

Treatment	% Infection
Isolated new blocks	4.3
Isolated recycled blocks	82.8
New-adjacent to recycled	56.4
Recycled-adjacent to new	71.5
New-adjacent to recycled (Blocked above table)	3.4

domycorrhizae

Microscopic observation of stained inoculated rootlets revealed successful mycorrhizal formation all inoculated redwood seedlings. Infection intensity ranged from small isolated vesicles to merous vesicles and arbuscles. Control plants displayed no sign of any endomycorrhizal formation.

gumes

Inoculated *T. subterraneum* seed germinated and rapidly outgrew competition to form dense mats. ter 1 yr about 50% average *T. subterraneum* cover was obtained. This increased to approximately the second year (Reed and Hektner 1983).

nomycete Nodulated Nitrogen Fixers

(a) Unnodulated nursery seedlings outplanted at Bridge Creek averaged 93% increase over initial ight during the first year. In the same time period nodulated seedlings average 526% increase.

(b) Nodulation was visible through the glass container 28 dys following inoculation. Two weeks er nodulation, sudden increases in leaf size and color were noted. Control plants, which never med nodules, became chlorotic and stunted. After 1 yr inoculated seedlings increased caliper by ree times and height by 12 times over that of the controls.

Table 2

Growth of Alders in Green House During First Year of Inoculation

	CALIPER			HEIGHT		
	Initial	1 Year	Increase	Initial	1 Year	Increase
Inoculated	2.3 cm	3.6 cm	57%	22.2 cm	39.8 cm	80%
Uninoculated	2.4	2.8	17	20.8	22.2	7

(c) Add seedlings inoculated with 0.080 g nodule/seedling or higher became nodulated. Below rate of inoculation nodules were not always formed. Inoculation of seed at 0.003 g seedling oduced higher rates of nodulation than inoculation of one month old seedlings.

Table 3

Percent Nodulation at Different Inoculation Rates

Treatment	% Nodulation	Age at Inoculation
Control	9	Seed
.001 g/tree	45	Seed
.003 g/tree	69	Seed
.003 g/tree	44	1 Month
.080 g/tree	100	1 Year
.125 g/tree	100	1 Year
.250 g/tree	100	1 Year

(d) Large scale alder inoculations resulting in nodulation produced seedlings with slightly larger calipers and tops. The main benefit was in overall vigor of seedlings. Leaf color was noticeably darker and stems green rather than red. Nodulation was present in 60-70% of the seedlings inoculated.

Table 4

Results of Large Scale Inoculations of Red Alder

Stock Type	Top Height	% Increase	Caliper	% Increase
Unnodulated	32.5 cm	-------	3.00 mm	-------
Nodulated	39.1	20.3	3.48	10.0
Well-developed nodules	42.9	32.0	4.25	41.7

(e) In the first 2 yrs following outplanting, mortality was reduced from 22% to 8% by nodulation in the nursery. Growth rate was improved in the first year and by an increased amount in the second. By the second year nodulated seedlings were strongly dominant over the unnodulated.

Table 5

Growth Rate and Mortality of Outplanted Alder

	Initial Ht.	% Ht. Incr. 1st Season	% Ht. Incr. 2nd Season	Mortality 1st Season	Mortality 2 Years
Unnodulated	39.1 cm	31.2%	83.2%	12%	22%
Nodulated	40.5	51.9	185.5	4	8

CONCLUSIONS

Both alder and clover were effectively established and rapidly became dominant on their appropriate sites. Growth was initially rapid, providing great competitive advantage on disturbed soils. This rapid growth is dependent on nitrogen fixing symbionts. Alder growth and survival was greatly reduced without nodules.

Mycorrhizal associations are essential to optimal growth and vigor of most plants. Without these associations plants have reduced chances of survival and growth. Stable long-term dominance is always dependent on mycorrhizae in the vegetation systems of Redwood National Park. Establishment of mycorrhizae on large quantities of trees is a difficult problem. Consistent establishment of *T. terrestris* on containerized Douglas-fir is an advantage over sterile seedlings. However, other fungal species are often desired but it is unlikely that they would be competitive under greenhouse conditions.

Establishment of *G.. fasiculatus* on *S. sempervirens* is an encouraging first step in the study on endomycorrhizal effect on growth and survival. The present state of knowledge concerning redwood endomycorrhizae is extremely limited.

Understanding the roles of symbiotic micro-organisms in natural and human disturbed ecosystems allows greater potential for successful recovery. A successful long-term restoration must rely on establishment of successional soil holding and building species. Rapid establishment and initial growth is essential for maximum effectiveness. Simultaneously establishing the components of a more stable, but slower growing system requires healthy, vigorous stock which will survive well in the initial years where vegetation is dominated by colonizers. Use of symbiotic micro-organisms to improve growth and survival will insure overall effectiveness of treatments.

MANAGEMENT RECOMMENDATIONS

Allowing successional vegetation types to naturally give way to stable systems will benefit long-term recovery. Management should take steps to accelerate and encourage succession but not circumvent it. Acceleration of processes can be achieved by the establishment of rapidly growing plants as soon as they will survive site conditions. This often involves simultaneous establishment of colonizers and potential conifer canopy species. Conifer stand composition can be manipulated by emphasizing species which are not expected to colonize effectively but are potentially important. Establishing naturally colonizing species is important when rapid establishment and initial growth are important.

The utilization of symbiotic micro-organisms in revegetation will not only achieve biological objectives but often will reduce overall costs. Inoculations of legume seed and red alder seedlings are extremely cost effective. Increased control of survival and growth rates will enable wider spacing and fewer plantings to achieve comparable results. Often results will be more desirable with fewer individuals growing to larger sizes. Unit cost for the inoculation of alder nursery stock was about 1.8% of the cost for seedlings. Considering expenses of planting labor and administration this is a very small percentage of overall expenses. Increased survival and growth rate results in a lower price for greater net benefit.

Naturally occurring vegetation is reliant upon micro-organisms and we must utilize them to effectively restore disturbed systems. At Redwood and other national parks we have the rare opportunity to naturally revegetate artificially disturbed systems.

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