relationships parallel the amount of LWD in the reaches, as the QU and EU sites contain the most. In contrast, the EL and QU sites are the only ones containing anadromous salmonids owing to blockage by the first dam. This raises the oft-discussed (and still unresolved) question by regional salmon and riparian scientists: "is it salmon and their marine-derived nutrients (MDN) or is it LWD that is the key ecosystem determinant in Pacific Northwest rivers?" It will require much more work on the microbial community to help address this question, though these preliminary results suggest the latter.

These preliminary results have provided a suite of potential microbial indicators of ecosystem condition and variability for long-term monitoring of the Elwha River before and after deconstruction of the dams in 2012; differences can be observed between the microbial community structures within the periphyton from the three different reaches of the Elwha River and from comparative reaches of the Quinault River. Consequently, this suite of tools will be further assessed on a larger scale via increased sampling over various seasons with the goal of establishing a permanent microbial community monitoring and assessment regimen for the Elwha Project. These approaches could also be used in other rivers undergoing long-term restoration activities, particularly those associated with dam removal and anadromous fisheries restoration efforts.

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Inoculation and Colonization of Four Saltmarsh Species with Vesicular-Arbuscular Mycorrhizal Fungi (Mississippi)

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The value of salt marshes in reducing wave energy, enhancing sedimentation, stabilizing sediment, providing fisheries habitat, and serving as a food source for wildlife is well documented and widely recognized. Vesicular-arbuscular mycorrhizal (VAM) fungi may have the potential to improve nursery production of native plants needed for saltmarsh restoration. The majority of work on mycorrhizal fungi has focused on terrestrial plants and this symbiosis is well understood and documented in these plants, in contrast to the role of VAM in salt marshes. Of the studies looking at saline marsh environments, most have been to determine the presence or absence of the fungi, and most of these studies have looked at naturally occurring fungi and not VAM introduced through commercially available inoculants. Vesicular-arbuscular mycorrhizal inoculation may increase plant growth, improve water transport, increase resistance to pathogens, and mediate transplant shock (McHugh and Dighton 2004). Healthier plants, with increased growth rates and a better ability to tolerate increased salinity and transplant stress and to acquire nutrients, could increase the effectiveness and success of saltmarsh restoration efforts.

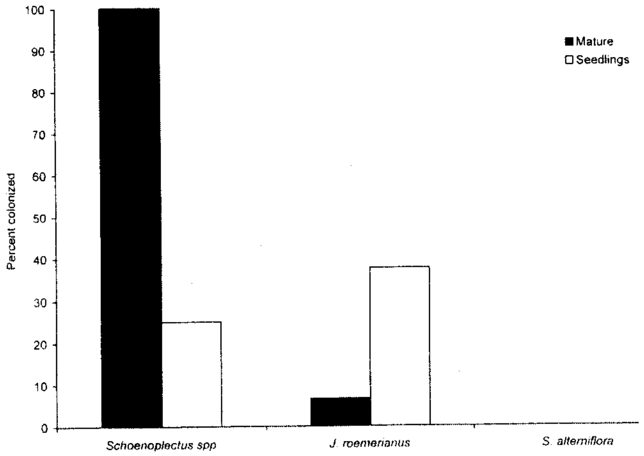

Figure 1. The percentage of plants that were colonized by vesicular-arbuscular mycorrhizae from a commercial inoculant in four species of graminoids at two life stages: mature and seedling (<6 months old): smooth cordgrass (*Spartina alterniflora*, mature *n* = 24, seedling *n* = 8), black needlerush (*Juncus roemerianus*, mature *n* = 22, seedling *n* = 24), three-square bulrush (*Schoenoplectus americanus*, mature *n* = 56), and saltmarsh bulrush (*S. robustus*, seedling *n* = 24).

In this study, we examined the colonization success of four species of saltmarsh graminoids common to the Mississippi coastline. We purchased a general endomycorrhizal inoculant (BioOrganics, La Pine OR) containing a blend of eight types of endospores in the genera *Glomus*, *Gigaspora*, and *Paraglomus*. We examined both mature and seedling stages of black needlerush (*Juncus roemerianus*), smooth cordgrass (*Spartina alterniflora*), three-square bulrush (*Schoenoplectus americanus*), and saltmarsh bulrush (*Schoenoplectus robustus*) for their ability to acquire VAM colonization. These species are typical of restorations in Mississippi and elsewhere in the Gulf of Mexico.

Seeds of black needlerush, saltmarsh bulrush, and smooth cordgrass were collected at the Gulf Coast Research Laboratory (GCRL), Ocean Springs, Mississippi, from wild populations during the spring and fall of 2007. After germination, seedlings were grown in peat pellets under controlled conditions until they were about 2–3 cm tall (6–8 weeks), when they were transferred to the greenhouse. Once seedlings of black needlerush and saltmarsh bulrush reached a height of 5–10 cm, they were planted in 10 cm pots (~600 cm^3) in 1:1 sand to topsoil mix. Smooth cordgrass seedlings were planted in 5 cm wells (~125 cm^3) in sterilized sand one month after germination. Sand had been sterilized in an electric soil sterilizer (Model SS15, Pro-Grow Supply, Broomfield WI) for two hours at 94°C.

Mature plants of smooth cordgrass and black needlerush were purchased from a nursery (Aquatic Plants of Florida, Sarasota) and planted in 15 cm pots (~1500 cm^3) in a mix of 1:1 potting soil to cow manure and held outdoors under a 55 percent shade cloth for four months before inoculation in late summer. Mature three-square bulrush plants were propagated from native rhizome buds collected at GCRL and rinsed free of sediments, then planted in 1:1 sand to topsoil mix and allowed to grow for six months before inoculation in 15 cm pots in the early spring. All pots were held in trays, which were kept more than half full of fresh water by watering at least once per week.

All mature plants received 5 cm^3 (~250 spores) of inoculant, introduced either as the raw powder or as a suspension in water to the top of the pot, thereby allowing spores to migrate down to the roots with routine watering. Seedlings of black needlerush and saltmarsh bulrush were inoculated with 5 cm^3 (~250 spores) by dusting the bottom of the peat pellets when they were stepped up to 10 cm pots. Seedlings of smooth cordgrass were inoculated with 0.25 cm^3 (~12 spores) of liquid suspension injected into the sand to the depth of the rhizosphere using a disposable pipette. The smaller volume used in the 5 cm wells standardized the amount of inoculant per unit volume of soil to keep this treatment comparable with that received by the other species in larger pots. An equal number of control plants were grown under identical conditions to the inoculated plants.

To confirm if colonization had occurred, we collected root samples 60–240 days after inoculation for all species. The roots of mature plants were gathered by lifting each plant out of the pot and snipping a small sample of roots from various locations around the root ball, inside and out, enough to fill a 20 ml scintillation vial halfway to the top; small, fine roots were preferred over larger, thicker roots. The roots of seedlings were gathered by removing the seedling from their pots and collecting the entire root mass. We used the staining technique by Vierheilig and others (1998) that uses hot 10% KOH and a solution of 5% black Sheaffer script ink in vinegar (5% acetic acid), an inexpensive, nontoxic alternative to more noxious stains. Colonization can be estimated in a multitude of ways after staining. Using the method by Vierheilig and others (1998), the chitin in the fungal cells stains blue-black while the plant material remains a reddish-brown, making colonization easy to distinguish. Stained roots were examined under a Zeiss Stemi dissecting scope at 20–50× magnification to determine the presence of fungal spores, hyphae, arbuscules, and vesicles. We scored each root sample as positive if fungi were present, irrespective of the quantity or type of colonization.

The mature black needlerush and smooth cordgrass plants showed little or no colonization. Only 6.6% of the black needlerush roots sampled were colonized, while none of the sampled smooth cordgrass roots were colonized. All of the sampled mature three-square bulrush plants showed colonization (Figure 1). Colonization was evident in 37.5% of black needlerush seedlings and 25.0% of the saltmarsh bulrush seedlings sampled, while smooth cordgrass seedlings had no observable colonized roots (Figure 1). Control plants sampled showed no indication of VAM colonization.

Our studies showed that black needlerush and bulrushes are capable of acquiring VAM colonization, although the rates of colonization were generally low and differed by species and age. Smooth cordgrass did not become colonized in either mature or seedling stages. It is interesting to note that percent colonization in these three genera appears to correlate with the zone occupied in Mississippi saltmarshes: smooth cordgrass is a low marsh species, black needlerush dominates the mid-marsh, and the bulrush species are restricted to the high marsh. Responses to VAM colonization can be mediated by plant species and environmental conditions. Allen and Cunningham (1983) concluded that the importance of VAM ranges from plants that are obligate mycorrhizal to nonmycorrhizal, whereas facultative mycorrhizal plants can differ greatly in their responses to VAM. Environmental factors that can affect colonization include flooding, tidal inundation, and soil composition (Hildebrandt et al. 2001), all of which vary from low to high marsh zones.

A second factor that could explain the rate and pattern of colonization we observed in the two bulrush species is the time of year that the experiments were conducted. Saltmarsh bulrush seedlings were examined during the winter months and were exposed to episodic freezing temperatures at night, whereas the mature three-square bulrush experiment was conducted during the summer months when plant growth is at its highest rate, and observed VAM colonization was also the greatest. This relationship between colonization and season might also have contributed to why black needlerush was only sporadically colonized; it is an extremely slow-growing plant and was sampled during the winter months when growth is at a minimum. Smooth cordgrass also exhibits clear seasonal growth patterns in the southeastern USA, increasing in biomass and shoot density during the spring and decreasing during late fall and early winter. Studies have shown that the degree of mycorrhizal colonization in salt marshes varies with season, both in intensity and in the formation of specific fungal structures (Boher et al. 2004). Furthermore, the degree of colonization is not constant during a plant's life cycle: colonization is at its greatest during vegetative growth and least during plant senescence (Hildebrandt et al. 2001).

A third factor that could have impacted the detection of infection is insufficient sampling of the root ball or the differences among species of VAM in the way that they accept staining. Intraradical hyphae vary considerably in morphology and architecture within the family of Glomales and only stain faintly, whereas Gigasporineae hyphae stain darkly (Mueller et al. 2004); both families were present in the commercial inoculant. There are conflicting studies on VAM and saltmarsh plants, with variation and inconsistency reported among and between species, suggesting that more research needs to be done to determine under what conditions mycorrhizal fungi are best able to colonize saltmarsh plants. Despite the difficulties with this initial series of experiments, we expect that under more ideal circumstances these species would benefit from VAM inoculation and provide practitioners with a supply of healthy, robust, native plants. We also recommend that native saltmarsh VAM and commercially available VAM should be compared when possible to determine if they differ in their colonization rates of saltmarsh plants. Future studies are needed to elucidate the most appropriate species, soil conditions, and timing of VAM introduction to benefit propagated plants in terms of increased growth, health, and ability to tolerate stressful conditions.

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Brassbuttons: An Introduced Species in a Restored Salt Marsh (Oregon)

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Estuarine wetlands along the Pacific coast have been dredged, diked, and drained for industrial, residential, and agricultural purposes. Because of the introduction of non-native species during the past centuries, it is unusual to find marshes with only native species. Several studies indicate that the disturbance of habitat favors the spread of non-native plants, which eventually outcompete the native flora, especially during prolonged low-salinity and soil-saturation periods (Kuhn and Zedler 1997).

Salt marsh restoration projects have typically included dike removal and short-term monitoring of plant communities. Over the years, soil subsides behind dikes and is compacted, especially if the areas are used for cattle grazing.