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SHORT NOTE

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The mycorrhizal colonization of six wetland plant species at sites differing in land use history

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Abstract Five wetland prairie sites and six native plant species in western Oregon were examined to determine patterns of vesicular-arbuscular mycorrhizal fungal (VAMF) colonization. The sites differed in type and intensity of past land use. VAMF colonization was tested *in situ* on seedlings from both field-sown seeds and from transplants. Colonization was measured as the percentage of root length with arbuscles or vesicles. All species (*Deschampsia cespitosa*, *Downingia elegans*, *Eriophyllum lanatum*, *Hordeum brachyantherum*, *Microseris laciniata*, and *Plagiobothrys figuratus*) became colonized by VAMF during the study. This is the first report of mycorrhizal colonization of these important native species. All sites supported mycorrhizal colonization of some of the experimental species. Average VAMF colonization ranged from 58% to 92% but was unrelated to subjective rankings of land use intensity. These results suggest that VAMF inoculum at all sites was sufficient to support revegetation by at least some species of native plants.

Key words Wetlands · Mycorrhizal fungi · Land use history · Restoration ecology · Soil ecology

Introduction

Many native wetlands have been disturbed by plowing, herbicide application, planting of agricultural crops, and invasion of non-native plant species. Intensive land uses such as these can destroy much of the soil's mycorrhizal fungal inoculum, preventing establishment and survival of mycorrhizal plants (Allen 1992; Cuenca and Lovera 1992; Ingham and Molina 1991; Jasper et al. 1989; Miller and Jastrow 1992). Loss of mycorrhizal inoculum can result in sites unsuitable for restoration of important native species, and may dictate the addition of mycorrhizal inoculum before restoration is possible (Miller and Jastrow 1992).

This study investigated the patterns of mycorrhizal colonization of important native wetland plants in sites which had undergone different types and intensities of past land use. We asked three specific questions: Are the study species mycorrhizal? Is spore number correlated with VAMF colonization? Is VAMF colonization related to intensity of past land use?

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Materials and methods

Study sites

Five sites in the southern Willamette Valley, Oregon were selected for study. These sites represent a diversity of past use practices, including tillage, application of agricultural chemicals, grazing, and solarization. The first study site ("Reed canarygrass") was a former monoculture of reed canarygrass (*Phalaris arundinacea* L.) in the process of weed control for restoration. The second site ("Ryegrass") was a former agricultural field tilled and herbicide treated for commercial ryegrass production. Restoration efforts at sites 1 and 2 started in 1990, including sowing with native grasses. The third site ("Solarized") was an area lightly pastured in the past but solarized for this study (6 m × 6 m plots tilled in May 1992 and sealed with clear 6 mm polyethylene over the summer). The fourth site ("Sod mat") was a former agricultural field that had received sections of soil with intact vegetation transferred in whole from a nearby high-quality wetland. The fifth site ("Abandoned pasture") was an unmanipulated area that had been lightly pastured in past decades; native grasses and forbs constituted about 45% of the plant cover at the time of the study (Wilson et al. 1995). Sites 1, 2, and 4 were near each other about 8 km west of Eugene, Oregon. Sites 3 and 5 were part of the same parcel about 13 km northwest of Eugene. All sites flood each winter and become droughty by the end of summer (Scoles Associates 1994).

We ranked the study sites by the likelihood that their land use histories would cause a depletion of VAMF inoculum (Table 1) using the following considerations. Repeated tilling and herbicide

Table 1 Vesicular-arbuscular mycorrhizal fungal (VAMF) colonization (percentage of root length with arbuscles or vesicles) for May and August sample dates, spore density in soil (number per g dry soil), and land use ratings (1 = most disruption of VAMF, 5 = least disruption of VAMF). Averages for species include only sites in which a species survived; averages for sites include only species that survived at that site (*NP* no plants survived for sampling on this date)

Species	Sampling date	Study sites					Average for species
		Reed canarygrass	Ryegrass	Solarized	Sod mat	Abandoned pasture	
Deschampsia cespitosa	May	NP	NP	NP	NP	NP	NP
	August	NP	60.9%	46.7%	NP	54.6%	54.1%
Downingia elegans	May	94.6%	85.6%	96.3%	NP	NP	92.1%
	August	100.0%	99.3%	98.9%	85.9%	54.9%	87.8%
Eriophyllum lanatum	May	NP	NP	50.4%	NP	73.1%	61.8%
	August	76.2%	NP	96.4%	NP	NP	86.3%
Hordeum brachyantherum	May	NP	NP	NP	NP	NP	NP
	August	71.1%	95.5%	72.9%	93.1%	86.4%	83.8%
Microseris laciniata	May	NP	NP	NP	NP	NP	NP
	August	100.0%	96.4%	100.0%	100.0%	85.0%	96.3%
Plagiobothrys figuratus	May	81.2%	87.7%	29.8%	NP	72.2%	67.7%
	August	97.1%	100.0%	99.7%	88.9%	85.8%	94.3%
Average for site	May	87.9%	86.6%	58.8%	NP	72.7%	76.5%
	August	88.9%	90.4%	85.8%	92.0%	73.3%	81.8%
Spore density		0.4	1.5	3.0	8.2	0.8	
Land use rating		1	2	3	4	5	

applications and long-term dominance by non-native plant species (sites 1 and 2) are detrimental to mycorrhizal fungi required by native species, as suggested in Allen (1992). Therefore, the monoculture of reed canarygrass at site 1 might select against a number of VAMF species. Intense soil solarization kills mycorrhizal spores and reduces plant root colonization potential (Afex et al. 1991; Nair et al. 1990). The soil disturbance in transferring the sod mats to site 4, though less severe than repeated tillage, could reduce VAMF numbers. Finally, the absence of soil tillage and the diversity of typically VAMF-colonized native plant species at site 5 would lead to the expectation that VAMF colonization would be most intact at this site.

Study species

Six herbaceous species were selected to represent plants typical of these wetlands habitats. *Deschampsia cespitosa* (L.) Beauv. and *Hordeum brachyantherum* Nevski are perennial grasses widespread in wetland prairies. No information on the mycorrhizal associations of these plants in the Pacific Northwest was available, although related European species are mycorrhizal (Harley 1959; Harley and Smith 1983). *Plagiobothrys figuratus* (Piper) Johnst. and *Downingia elegans* (Dougl.) Torr. are annual forbs. *Microseris laciniata* (Hook.) Schultz-Bip. is a perennial, rosette forb. These three species are not reported in the mycorrhizal literature. *Eriophyllum lanatum* (Pursh) Forbes is a subshrub, whose closest relative reported in European literature is not mycorrhizal (Harley 1959).

Plant establishment

In October 1992, 60 seeds per species were sown into 0.125 m^2 areas cleared of existing vegetation at each study site. The plots remained cleared of competing vegetation for the study period, except at the Abandoned-pasture and Sod-mat sites. At these sites, cleared plants regrew from surviving roots and rhizomes. Therefore, competition of the experimental seedlings with other plants was probably highest at these two sites.

Seeds were covered with plot soil. Establishment under field conditions was low for all species except *Plagiobothrys figuratus*. Additional experimental plants were germinated on sterile agar in the laboratory and transplanted into the site in May 1993, when the seedlings were 2–3 cm in height.

Spore density

The number of VAMF spores per gram of soil was determined (see Ianson and Allen 1986) by mixing 5 g of soil with water in a centrifuge tube to a final volume of 12 ml. After letting the soil hydrate for 15 min, the sample was centrifuged at 2000 rpm for 10 min. The pellet containing the VAMF spores was resuspended in a 2 M sucrose-2% Calgon solution, and centrifuged again for 10 min at 2000 rpm. The resulting liquid was decanted into a 125-ml separatory funnel, letting the liquid slowly drain from the separatory funnel (1 drop every 5 s). The VAMF spores adhered to the sides of the glass funnel and were obtained by rinsing the sides of the funnel with water into a beaker. VAMF spores in the suspension were counted under a dissecting microscope.

Mycorrhizal colonization of roots

We used the *in situ* method of measuring VAMF plant colonization potential, because it measures actual colonization under field conditions. Roots for assessing the VAMF colonization of each species were obtained from the six largest plants growing in each plot on each of the two sample dates (early May 1993 and early August 1993). The May sample was of seedlings from field-sown seed; the August sample from transplanted seedlings (except for *Plagiobothrys figuratus*, in which plants collected in August were from germinated seeds that survived the entire summer). Plants were collected by carefully loosening the soil around the roots of each plant and lifting the roots gently from the soil. Roots were shaken to remove adhering soil and placed in labeled plastic zip-lock plastic bags. Soil from around the plant roots was placed in another appropriately labeled zip-lock plastic bag for determination of spore density.

In the laboratory, colonization was determined by clearing and staining (McGonigle et al. 1990): 2-cm root segments were cleared by placing in 10% KOH, removed from the KOH, rinsed in tap water, and placed in trypan blue-lactic acid stain for 0.5–2 h to allow vesicular-arbuscular mycorrhizal structures to stain clearly in the roots. Roots were rinsed in distilled water and stored in lactic acid until observed.

We measured at least 50 cm of total root length from each of 12 plants. The root length occupied by vesicules or arbuscules was measured using a dissecting microscope, and colonization defined as the proportion of root length that these structures covered. We differentiated mycorrhizal structures from other types of fungi that colonize roots by not accepting hyphal material alone as evidence for mycorrhizal colonization.

Results and discussion

Are the study species mycorrhizal?

All six species became colonized by VAMF during the study. Proportion of root length colonized, averaged across all sites, ranged from 54% for *Deschampsia cespitosa* and 96% for *Microseris laciniata* (Table 1). Except for the low rate of colonization of *Plagiobothrys figuratus* at the Solarized site (30%), site colonization rates on surviving seedlings and transplants were all above 46%. This is the first documentation of mycorrhizal colonization of these six important native species.

Was spore number correlated with colonization?

VAMF spores were recovered from each site (Table 1), showing that each site had the potential for mycorrhizal colonization. Spore density varied 20-fold among sites, from 0.4 per g dry soil at the Reed-canarygrass site to 8.2 per g dry soil at the Sod-mat site. This variation was unrelated to our land-use rating (Kendall rank correlation, $P>0.20$), and did not translate into differences in average mycorrhizal colonization (Kendall rank correlation, $P>0.20$) on the experimental plant species.

Elevated temperatures under solarized treatments in agricultural fields are thought to kill fungal spores associated with plant diseases. Yet spore concentration was 3.5-fold higher at the Solarized site than at the adjacent unmanipulated site. Thus, solarization conditions were probably not extreme enough, even under the relatively hot and dry weather in 1992, to reduce spore densities. Solarization also failed to reduce the soil seed bank (Wilson et al. 1995).

Was VAMF colonization related to intensity of past land use?

All sites supported mycorrhizal colonization of at least four of the six experimental species. Moreover, at all sites except the Abandoned-pasture site, overall VAMF colonization of the experimental species was very high (averaging >85% of the root lengths sampled in August, Table 1).

Sites differed in the degree to which individual species became colonized by VAMF. For example, VAMF colonization of *Plagiobothrys figuratus* seedlings in May were lowest at the Solarized site, whereas colonization of *Downingia elegans* seedlings were lowest at the Abandoned-pasture site.

Average root colonization by VAMF did not match our index of land-use intensity (Kendall rank correlation, $P>0.20$). Either past land use was not severe enough to reduce VAMF inoculum, or more recent restoration activities at these sites had allowed reestablishment of VAMF inoculum. Although all sites had adequate mycorrhizal inoculum to support regrowth of at least several native plant species, differences between sites and species show that uniform restoration results should not be expected.

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