

## MYCORRHIZAL COLONIZATION ACROSS HYDROLOGIC GRADIENTS IN RESTORED AND REFERENCE FRESHWATER WETLANDS

Candice R. Bauer<sup>1,2</sup>, Chev H. Kellogg<sup>1,3</sup>, Scott D. Bridgham<sup>1,4</sup>, and Gary A. Lamberti<sup>1</sup>

<sup>1</sup>*Department of Biological Sciences*

*University of Notre Dame*

*Notre Dame, Indiana, USA 46556-0369*

<sup>2</sup>*Present addresses:*

*USEPA Region 5, WQ-16J*

*77 West Jackson Street*

*Chicago, Illinois, USA 60604*

*E-mail: bauer.candicer@epa.gov*

<sup>3</sup>*USGS Science Center*

*3200 SW Jefferson Way*

*Corvallis, Oregon, USA 97331*

<sup>4</sup>*Department of Biology*

*1210 University of Oregon*

*Eugene, Oregon, USA 97403-1210*

**Abstract:** Arbuscular mycorrhizae, which are plant root-fungal symbioses, are common associates of vascular plants. Such relationships, however, are thought to be rare in wetland plant roots, although several recent studies suggest that arbuscular mycorrhizae may be important in wetland ecosystems. Our objectives were to determine (1) the level of arbuscular mycorrhizal colonization of plant roots in three freshwater marshes and (2) the effect of restoration status, hydrologic zone, and plant species identity on mycorrhizal colonization. We quantified the percentage of plant roots colonized by mycorrhizal fungi in one reference and two restored freshwater marshes in northern Indiana, USA during summer 1999. Roots were collected from soil cores taken around dominant plant species present in each of three hydrologic zones and then stained for microscopic examination of mycorrhizal colonization. Mycorrhizae were present in each wetland, in all hydrologic zones and in all sampled plants, including *Carex* and *Scirpus* species previously thought to be non-mycorrhizal. Both restored and reference wetlands had moderate levels of mycorrhizal colonization, but no clear trends in colonization were seen with hydrologic zone, which has been hypothesized to regulate the formation of mycorrhizae in wetlands. Mycorrhizal colonization levels in the roots of individual species ranged from 3 to 90% and were particularly large in members of the Poaceae (grass) family. Our results suggest that arbuscular mycorrhizae may be widely distributed across plant species and hydrologic zones in both restored and reference freshwater marshes. Thus, future research should examine the functional role of mycorrhizal fungi in freshwater wetlands.

**Key Words:** arbuscular mycorrhizal fungi, mycorrhizae, freshwater wetlands, restoration, plant-fungal interaction

### INTRODUCTION

Plant root-fungal interactions, called mycorrhizae, are found in approximately 90% of all vascular plants (Allen 1991). Arbuscular mycorrhizae clearly play important roles in terrestrial ecosystems, such as grasslands, where they influence plant community structure and nutrient cycling (Jackson and Mason 1984). In contrast, the role of mycorrhizae in wetland plant communities is controversial because mycorrhizal fungi are believed to be un-

able to survive the anaerobic conditions present in wetland soils (Miller 2000). Some recent studies, however, have reported the presence of arbuscular mycorrhizal fungi in wetland habitats (e.g., Wetzel and van der Valk 1996, Cooke and Lefore 1998, Turner and Friese 1998), although their ubiquity and function in wetlands is unclear. In this paper, we compare the level of mycorrhizal colonization in plant roots of both restored and reference freshwater marshes in Indiana, USA.

Functional relationships are formed between plants and arbuscular mycorrhizal (AM) fungi when AM fungi invade the cortical cells of plant roots. Specialized nutrient-transfer structures known as arbuscules are formed within the root, as well as intracellular fungal storage vesicles (Allen 1991, Sharma et al. 1997). The large hyphal network present in the soil allows the plant to access water and nutrients otherwise unavailable because they are outside the root zone (Jackson and Mason 1984). Hyphae in the soil transport nutrients, particularly phosphorus, to plant roots faster than such nutrients can move via diffusion in the soil alone. In return, AM fungi receive carbon from the plant as photosynthates or in the form of carbon-rich root exudates (Allen 1991, Harley 1991). This relationship is often considered mutualistic, with mycorrhizal plants having greater growth that can affect competitive interactions among plants (Hetrick et al. 1994), as well as community structure (Brundrett 1991, Francis and Read 1994) and plant biodiversity (Ozinga et al. 1997, van der Heijden et al. 1998, Hartnett and Wilson 1999). Further, plant-mycorrhizal relationships can influence ecosystem processes such as carbon, nitrogen, and phosphorus cycling (Abbott and Robson 1991, Allen 1991).

While prairie plants, particularly members of the Poaceae or grass family, may have 70% to 80% of their root length heavily colonized by AM fungi (Read et al. 1976), wetland plants traditionally have been thought to have little or no root colonization. For example, many common wetland plant families, most notably the Cyperaceae or sedges, had been previously categorized as non-mycorrhizal because few species' roots were observed to harbor the fungus (Khan 1974, Powell 1975). Additionally, few species of semi-aquatic plants have been found to host AM fungi. For example, only 9 of 49 species examined in Denmark aquatic habitats were mycorrhizal (Beck-Nielsen and Madsen 2001) and none of the five herbaceous plants sampled in Alberta marshes were mycorrhizal (Thormann et al. 1999).

In contrast, several other studies have shown that wetland plants, including the sedges (Miller et al. 1999), can have significant mycorrhizal colonization. Mycorrhizal colonization has been found in wetland plants from numerous habitats, including salt marshes (Cooke and Lefor 1990, Wigand and Stevenson 1994, Brown and Bledsoe 1996), prairie potholes (Wetzel and van der Valk 1996), prairie fens and other groundwater-fed wetlands in the midwestern United States (Turner and Friese 1998, Turner et al. 2000), floating wetland mats (Stenlund and Charvat 1994), and the Everglades (Aziz et al. 1995). Therefore, the literature on wetland plant mycorrhizae is incomplete and often contradictory.

Our primary objective was to determine if colonization by AM fungi differed between restored and reference wetlands across three hydrologic zones. We expected that mycorrhizal colonization would be found in each of the wetlands but that levels in the restored wetlands would be lower than in reference sites. For example, disturbance can greatly reduce the inoculum potential of the soil (Reeves et al. 1979, Allen and Allen 1990), which can result in disturbed sites having lower levels of root colonization by AM fungi than undisturbed sites, as has been demonstrated in salt marshes (Cooke and Lefor 1990). Alternatively, colonization could be greater in restored wetlands than in reference wetlands because some disturbed wetlands have been found to have greater levels of mycorrhizal colonization than reference sites (Aziz et al. 1995, Turner and Friese 1998). We also expected colonization levels to be inversely related to soil moisture in wetlands (Stevens and Peterson 1996, Miller 2000) and that the different species of plants would have different levels of mycorrhizal colonization. Specifically, we predicted that wetland grasses would have the highest level of root colonization by AM fungi (Wetzel and van der Valk 1996).

## MATERIALS AND METHODS

### Site Description

During the 1999 growing season, we sampled three freshwater marshes in northern Indiana that were isolated from other surface waters (41°30'–41°32'N, 86°20'–86°26'W). Two of the wetlands were restored in 1994 (Restored 1 and Restored 2; each ~2 ha), while the third wetland (Reference; ~7 ha) had not been previously drained or cultivated. The restored wetlands had been drained and were cultivated for at least 100 years prior to restoration, when drainage tiles in the area were removed in 1994. No seeding or planting was performed on either restored wetland. Vegetation of the two restored wetlands was dominated by *Leersia oryzoides*, *Scirpus cyperinus*, *Typha* spp., and *Phalaris arundinacea*, while the reference wetland was dominated by *Calamagrostis canadensis*, *Carex lasiocarpa*, *Phalaris arundinacea*, and *Typha* spp. (Kellogg and Bridgham 2002).

Nutrients were analyzed from cores collected from the saturated and submerged zones of each wetland in July 1998. The top 10 cm of soil, with the litter layer removed, were analyzed for particle size using the hydrometer method (Sheldrick and Wang 1993), total percent carbon (Tiessen and Moir 1993) and nitrogen (McGill and Figueiredo 1993) using a Perkin-Elmer 2400 CHN analyzer, and total percent phosphorus by perchloric digestion (Lachat BD-46 di-

gester; O'Halloran 1993) followed by analysis with a Lachat Quikchem 8000 autoanalyzer.

The restored wetlands had mineral soils (Restored 1: 27.5% clay, 27.5% sand, and 45% silt; Restored 2: 30% clay, 40% sand, and 30% silt) and similar amounts of carbon, nitrogen, and phosphorus (Restored 1: 1.9%, 0.27%, and 0.5%, respectively; Restored 2: 2.4%, 0.33%, and 0.4%, respectively). In contrast, the reference wetland was a histosol (Houghton muck) and had higher percentages of carbon and nitrogen (25.9% and 2.54%, respectively) than the restored wetlands. Percent total phosphorus in the reference wetland (0.2%) was lower than in the restored wetlands.

### Sampling Procedures

Three permanent transects, ranging in length from 14 to 24 m, were established perpendicular to the shoreline in each wetland and subdivided into 3 hydrologic zones. The summer of 1999 was unusually dry, and therefore, hydrologic zones were based largely on the vegetation. The submerged zone was estimated by the presence of mudflat annuals and emergent macrophytes such as *Alisma plantago-aquatica*. The wetland-upland border area or border zone was delineated by the presence of dry soil conditions and encroachment of *Solidago* sp., including *S. canadensis* L., *S. patula* Muhl., and *S. riddellii* Frank, which are typical of wet prairies (Voss 1996). The saturated zone contained the sedge-dominated area between the submerged and the border zones. Sampled plants were chosen from the central portion of each zone to minimize error in zone delineations. Visits to these sites in 2000, a wetter year, confirmed the validity of our 1999 zone assessments.

We sampled the two restored wetlands on 15–16 June 1999 and all three wetlands on 3–5 August 1999. Within each zone, soil cores of 10-cm diameter and approximately 15-cm depth were taken from each of the dominant plant species by placing a PVC corer around the base of the plant. Three dominant plant species were sampled in most zones, resulting in approximately 27 cores per wetland. Soil cores were placed in ziplock bags, returned to the laboratory, and refrigerated until the roots were collected from the soil. A subsample of each core was weighed, dried at 80°C for at least 48 hours, and reweighed to determine percent soil moisture. Plants were identified using Voss (1972, 1985, 1996), and verified using species lists from previous work in these wetlands (C. H. Kellogg, unpublished data) if no fruit were present.

### Mycorrhizae Quantification

Root segments that were attached to the plant stem were removed from the soil core and placed in 50% ethanol until staining. The roots were cleared overnight using 10% KOH, decolorized in alkaline hydrogen peroxide (20–40 min), acidified with 10% hydrochloric acid for 40 min, and stained with 0.05% trypan blue in acidified glycerol at room temperature (modified from Kormanik and McGraw 1982, Koske and Gemma 1989). The stained root sections were stored in acidified glycerol until analyzed.

Mycorrhizae in root sections were quantified using the grid-intersect method (Giovannetti and Mosse 1980) for 1-cm-long root sections laid randomly in a gridded petri dish. Each grid-intersection was categorized for the presence of mycorrhizal fungi, as arbuscules, vesicles, and hyphae, using a dissecting microscope (10–100 X). If only hyphae were present in the intersection, but arbuscules or vesicles were present in the field of view, this intersection was included in the "hyphae only" category (McGonigle *et al.* 1990). The percent of intersections with colonization by mycorrhizal fungi was calculated by adding the number of intersections with arbuscules, vesicles, and hyphae only and then dividing the sum by the total number of intersections scored.

### Statistical Analyses

To determine whether mycorrhizal colonization differed over the growing season, we used a t-test to compare the percent of root intersections colonized by mycorrhizal fungi for the June and August collections in the two restored wetlands. Because differences were significant ( $p=0.007$ ), we then conducted a 2-way ANOVA to test for the effects of wetland and hydrologic zone on mycorrhizal colonization within each sampling date. A 1-way ANOVA or t-test was used to determine if common taxa ( $n \geq 4$ ) or families (Poaceae and Cyperaceae), respectively, had different levels of mycorrhizal colonization. We conducted multiple contrast tests (Tukey's HSD) when three or more groups were present in a significant analysis. When necessary, data were log-transformed prior to analysis to meet the statistical assumptions of ANOVA. Non-parametric correlation was used to determine if soil moisture and percent colonization were related over all sampled cores because the data were not normally distributed.

## RESULTS

### Mycorrhizal Colonization Over Time

Arbuscular mycorrhizal fungi were found in plant roots during both June and August sampling periods

Table 1. Plant taxa collected, number of samples collected (n), collection sites (where 1 = Restored 1, 2 = Restored 2, and R = Reference wetland), observed range of mycorrhizal colonization found in our study, and ranges cited in the literature.

Family	Species	n	Wetland			Mycorrhizal Colonization (%)	Cited Ranges (%) <sup>*</sup>
			1	2	R		
Alismataceae	<i>Alisma plantago-aquatica</i> (L.)	4	X			13–37	0 <sup>7</sup>
Asclepiadaceae	<i>Asclepias incarnata</i> (L.)	2	X			13–57	80 <sup>4</sup>
Asteraceae	<i>Cirsium</i> sp.	2			X	38–81	—
Asteraceae	<i>Sagittaria latifolia</i> Willd.	3			X	18–30	4–11 <sup>3</sup>
Asteraceae	<i>Solidago</i> sp.	2		X		62–70	—
Cyperaceae	<i>Carex cristatella</i> Britton	2		X		9–44	30 <sup>5</sup>
Cyperaceae	<i>Carex lasiocarpa</i> Ehrh.	4			X	3–34	0 <sup>8</sup> , 4–9 <sup>3</sup>
Cyperaceae	<i>Carex lurida</i> Wahl.	6		X		19–47	60–80 <sup>4</sup>
Cyperaceae	<i>Carex tribuloides</i> Wahlenb.	2	X	X		21–27	50 <sup>4</sup>
Cyperaceae	<i>Carex vulpinoidea</i> Michau	8	X	X		8–80	0–80 <sup>4</sup> , 28 <sup>5</sup>
Cyperaceae	<i>Scirpus acutus</i> Bigelow	5	X		X	9–43	0–12 <sup>3</sup>
Cyperaceae	<i>Scirpus atrovirens</i> Willd.	2		X		20–21	1–14 <sup>3</sup> , 20–38 <sup>6</sup> , 90 <sup>4</sup>
Cyperaceae	<i>Scirpus cyperinus</i> (L.) Kunth	9	X	X		3–51	10–80 <sup>4</sup>
Cyperaceae	<i>Scirpus validus</i> Vahl	11	X	X		3–85	NA
Poaceae	<i>Leersia orzoides</i> (L.) Sw.	9	X	X		19–74	0–100 <sup>4</sup>
Poaceae	<i>Panicum clandestinum</i>	2		X		41–62	NA
Poaceae	<i>Phalaris arundinacea</i> (L.)	18	X	X	X	3–90	0 <sup>7</sup> , 0–24 <sup>2</sup> , 21 <sup>1</sup> , 80 <sup>4</sup>
Polygonaceae	<i>Polygonum</i> sp.	2	X			7–40	—
Potamogetonaceae	<i>Potamogeton natans</i> L.	6	X			11–84	0 <sup>7</sup>
Sparganiaceae	<i>Sparganium chlorocarpum</i> Rydb.	2		X		6–18	NA
Typhaceae	<i>Typha</i> spp.	10	X	X	X	4–54	—

\* 1 = Read et al. (1976), 2 = Rickerl et al. (1994), 3 = Wetzel and van der Valk (1995), 4 = Cooke and Lefor (1998), 5 = Miller et al. (1999), 6 = Turner et al. (2000), 7 = Beck-Nielsen and Madsen (2001), and 8 = Cornwell et al. (2001). — refers to plant taxon that was not identified to species and thus we could not list cited ranges. NA is listed when, to our knowledge, no previous record of colonization was found for this species.

in each of the wetlands. Overall, mycorrhizal colonization in the restored wetlands was significantly greater in June ( $41 \pm 3\%$ , mean  $\pm$  1SE) than in August ( $28 \pm 4\%$ , t-test  $p = 0.007$ ). Furthermore, we found evidence of mycorrhizal colonization in roots of each sampled plant, including species that previously have

not been reported as mycorrhizal (Table 1; as denoted by NA or 0 in cited ranges column). For example, we found that *Alisma plantago-aquatica*, *Scirpus validus*, *Panicum clandestinum*, and *Sparganium chlorocarpum* had 18–85% of their root length colonized by arbuscular mycorrhizal fungi. The range of colonization in our study was broadly similar to that reported in several recent papers investigating the role of mycorrhizal fungi in freshwater wetlands (Table 1).

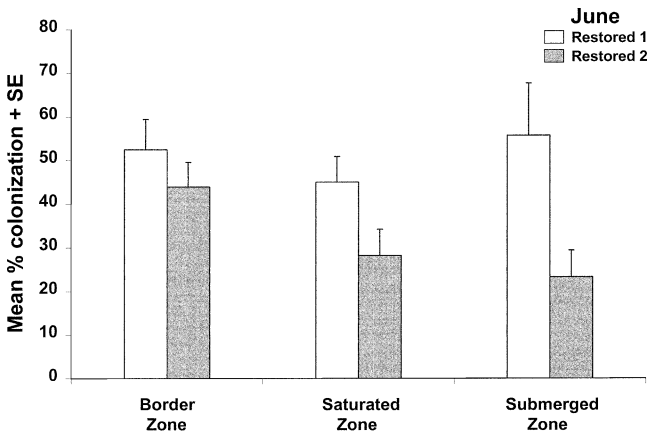


Figure 1. Arbuscular mycorrhizal colonization of plant roots in two restored Indiana wetlands by hydrologic zone in June 1999. Restored 1 had significantly greater AM colonization than Restored 2, but no significant differences were found between hydrologic zones.

#### Influence of Wetland and Hydrologic Zone

In June, Restored 1 had a significantly greater level of mycorrhizal colonization than Restored 2 (ANOVA:  $F_{1,41} = 11.536$ ,  $p = 0.002$ ), but hydrologic zones did not differ (ANOVA:  $F_{2,41} = 1.595$ ,  $p = 0.215$ ; Figure 1). In Restored 2, however, mycorrhizal colonization tended to increase with distance from standing water (Figure 1). Wetland and hydrologic zone did not interact significantly (ANOVA:  $F_{2,41} = 1.385$ ,  $p = 0.262$ ).

In August, wetland, hydrologic zone, and their interaction all had significant effects on overall mycorrhizal colonization (ANOVA: Wetland  $F_{2,63} = 4.051$ ,  $p = 0.022$ ; Zone  $F_{2,63} = 13.769$ ,  $p < 0.001$ ; Wetland\*Zone  $F_{4,63} = 3.303$ ,  $p = 0.016$ ; Figure 2). In the

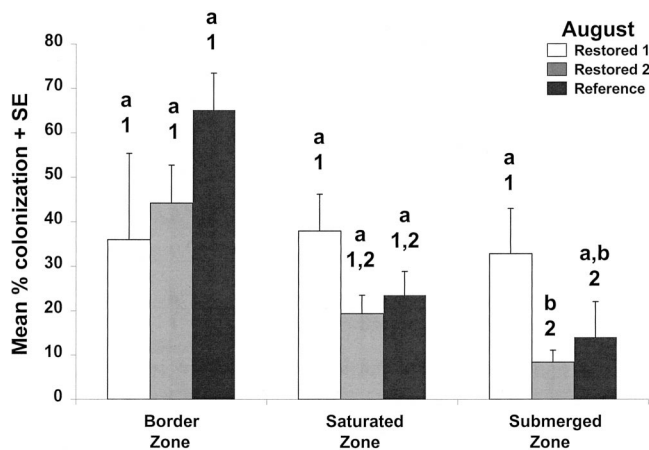


Figure 2. Arbuscular mycorrhizal colonization of plant roots in two restored Indiana wetlands and one natural wetland by hydrologic zone in August 1999. Different letters indicate significant differences among wetlands within a particular zone; different numbers indicate significant differences among zones within a wetland (Tukey's test,  $p < 0.05$ ).

border zone, the reference wetland tended to have greater colonization levels than the two restored wetlands, but this trend was not significant. Restored 1 tended to have greater colonization levels than the other two wetlands in both the saturated and submerged zones. In particular, Restored 1 had a greater colonization level than Restored 2 in the submerged zone (Tukey's  $p < 0.01$ ; Figure 2). Considering hydrologic zones within each wetland, Restored 1 had similar colonization levels across all hydrologic zones, ranging between 30% and 40%. In contrast, Restored 2 had significantly greater colonization levels in the wetland-upland border area than the submerged zone (Tukey's  $p < 0.01$ ). The reference wetland also had significantly greater colonization levels in the border zone than the submerged zone (Tukey's  $p < 0.01$ ). In general, Restored 2 and Reference showed a trend of decreasing colonization levels with increasing soil moisture. However, using all the cores collected, the level of mycorrhizal colonization was not correlated with the percent soil moisture (Spearman's  $r = 0.049$ ,  $p > 0.5$ ,  $n = 93$ ).

Overall, differences in colonization determined for the restored and reference wetlands in August were not due to the restoration status of the wetlands. Specifically, the Reference wetland had levels of mycorrhizal colonization intermediate between Restored 1 (greatest overall colonization) and Restored 2 (lowest overall colonization) when all plant taxa were examined. A similar pattern was found in the level of colonization for the two plant species common to all wetlands, *Typha* spp. and *Phalaris arundinacea*. Both *Typha* and *Phalaris* (determined using all hydrologic zones combined) had the greatest levels of mycorrhizal coloni-

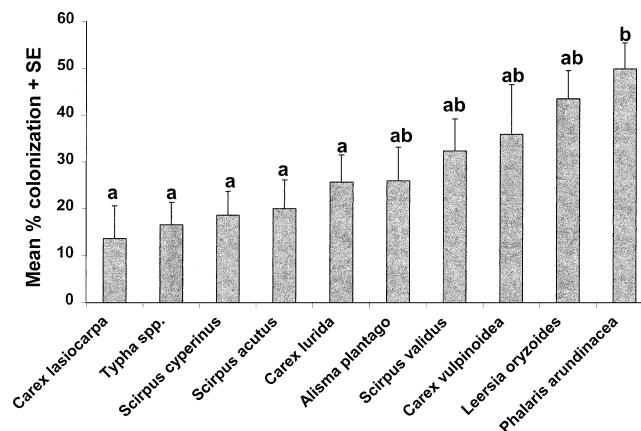


Figure 3. Arbuscular mycorrhizal colonization of common plant taxa collected from three Indiana wetlands during the 1999 growing season ( $n \geq 4$  per plant taxon). Different letters indicate significant differences among plant taxa (Tukey's test,  $p < 0.05$ ).

zation in Restored 1 (*Typha*: 53.7%,  $n = 1$ ; *Phalaris*:  $60.8 \pm 11.6\%$ ,  $n = 4$ ), intermediate levels of colonization in Reference (*Typha*:  $20.7 \pm 5.7\%$ ,  $n = 2$ ; *Phalaris*:  $52.2 \pm 9.7\%$ ,  $n = 6$ ), and the lowest levels of colonization in Restored 2 (*Typha*:  $9.8 \pm 4.8\%$ ,  $n = 3$ ; *Phalaris*:  $9.2 \pm 6.4\%$ ,  $n = 2$ ).

#### Influence of Plant Taxon

Mycorrhizal colonization levels differed across plant taxa (ANOVA,  $F_{29,89} = 2.568$ ,  $p < 0.001$ ; Figure 3). For example, members of the Cyperaceae family, such as *Carex* spp. and *Scirpus* spp., as well as emergent wetland plants including *Typha* spp. and *Alisma plantago-aquatica*, had levels of average mycorrhizal colonization ranging between 14% (*Carex lasiocarpa*) and 36% (*Carex vulpinoidea*). In contrast, grasses (Poaceae) such as *Phalaris arundinacea* and *Leersia oryzoides* had average colonization levels of 50% and 43%, respectively. Overall, Poaceae family members had significantly greater colonization levels ( $49.4 \pm 3.9$ ) than did Cyperaceae family members ( $26.6 \pm 2.8$ ; t-test  $p < 0.001$ ).

## DISCUSSION

We found evidence of arbuscular mycorrhizae in all three freshwater marshes and AM colonization of all sampled wetland plant taxa. Several of these taxa, including *Alisma plantago-aquatica*, *Scirpus validus*, *Panicum clandestinum*, *Sparganium chlorocarpum*, and *Potamogeton natans*, to our knowledge have not previously been reported to host AM fungi. While some of the plants sampled in our study, such as *Carex lasiocarpa* and *Scirpus cyperinus*, had low average

levels of AM fungal colonization, other plants including *Scirpus validus*, *Carex vulpinoidea*, and *Phalaris arundinacea* had 32 to 50% of their root length colonized by AM fungi. Individual root segments sometimes had AM colonization of 80 to 90%. The overall range of colonization recorded in this study was consistent with the levels recorded in other studies, further supporting the prevalence and potential importance of mycorrhizal fungi in both restored and reference wetland ecosystems.

Differences in AM colonization were observed at the taxonomic level and at the wetland scale, particularly in June, but generally not across hydrologic zones. At the taxonomic level, *Phalaris arundinacea* had significantly greater colonization than several *Carex* and *Scirpus* species. These differences could be due to (1) the plants' evolutionary histories that have led to differing degrees of dependence on AM fungi, ranging from obligate to facultative interactions, (2) differing root morphologies that encourage or limit AM colonization, or (3) species-specific differences in nutrient and water-level optima (Allen 1996). Regardless, every wetland plant species that we inspected had some level of AM colonization, suggesting that these plant-fungal relationships may be common in wetlands.

Overall, AM colonization levels did not vary significantly between the restored and reference wetlands, with greater differences between the two restored wetlands than between the restored and reference wetlands. However, the wetlands had different plant species compositions, which could have influenced the overall level of mycorrhizal colonization determined for each wetland. Therefore, to compare the level of colonization among the wetlands better, mycorrhizal colonization of the common taxa *Phalaris arundinacea* and *Typha* spp. across the three wetlands were assessed. This analysis allowed us to determine if restoration history affected AM colonization, without confounding the results by comparing wetlands having different species compositions. For both *Phalaris* and *Typha*, the level of AM colonization in the reference wetland fell between the levels of the two restored wetlands. Thus, our results suggest that similar levels of mycorrhizal colonization, as determined by both overall and species-specific colonization levels, may be found in restored and reference wetlands.

Location along a hydrologic gradient and associated differences in soil moisture have also been hypothesized to be important factors in mycorrhizal colonization (Stevens and Peterson 1996, Miller 2000). In our study, there were no clear patterns in overall colonization across hydrologic zones. This finding contrasts with recent literature suggesting that soil wetness is an important factor regulating mycorrhizal coloni-

zation in Carolina depressional wetlands (Miller 2000) and conventional wisdom, which suggests that mycorrhizal fungi cannot survive in the anoxic soils often present in wetlands (Aziz et al. 1995, Brown and Bledsoe 1996). However, analyses of AM colonization for individual species over a soil moisture gradient or over a longer time frame (e.g., annual cycle) could lead to different results. Our results may have been influenced by the dry conditions of summer 1999. However, Wetzel and van der Valk (1996) proposed that soil moisture may not be as important as previously thought because they found similar high levels of mycorrhizal colonization in wetland plant roots and in drier upland zones during a wet year. These findings, combined with our results showing that overall levels of mycorrhizal colonization were not correlated with hydrologic zone or percent soil moisture at the time of collection, suggests that soil moisture is not solely responsible for the levels of mycorrhizae in wetland plant roots.

On the larger scale, Turner et al. (2000) suggested that differences in colonization among wetlands are related to physico-chemical differences. For example, physico-chemical differences in soil pH, phosphorous, and specific conductance were important in determining the level of arbuscular mycorrhizal colonization in prairie potholes (Wetzel and van der Valk 1996). While physico-chemical factors were likely important in our study (i.e., the two restored wetlands had different levels of AM colonization), they did not exclude mycorrhizal formation. Rather, the unpredictability of mycorrhizal colonization in wetlands is likely due to the numerous factors controlling the formation of mycorrhizae, including the identity and nutritional status of the host plant, fungus abundance and identity, and the soil or environmental conditions at the site (Brunnett 1991).

The functional role of mycorrhizae in wetlands is only just beginning to be understood. Mycorrhizae are thought to have a functional role in phosphorus cycling in wetlands, as they do in upland systems (White and Charvat 1999, Cornwell et al. 2001, Tang et al. 2001). An emergent aquatic plant (*Lythrum salicaria* L.), for example, was colonized by arbuscular mycorrhizae only when the phosphorus concentration in soil pore water was below 1 mg/L (White and Charvat 1999). *Typha angustifolia* L. was colonized in three low-phosphorus treatments but not at the highest phosphorus treatment (Tang et al. 2001). The level of colonization decreased when phosphorus was added to *Solidago patula* Muhl. (Cornwell et al. 2001), and increased phosphorus uptake was documented in a semiaquatic grass colonized with mycorrhizal fungi (Miller and Sharitz 2000). In our study, the levels of total phosphorus (ranging from 0.1 to 0.6%) were ei-

ther not large enough to exclude mycorrhizal colonization completely, or mycorrhizae were transporting other limiting elements to the plants.

Given the potential importance of mycorrhizae in wetland ecosystems, coupled with their structuring role in better studied terrestrial systems, we should consider the role of AM fungi in wetland restoration. In our study, we found that restored and reference wetlands had similar, moderate levels of mycorrhizal colonization. In contrast, vegetation in a restored saltmarsh lacked AM fungi (Cooke and Lefor 1990). Since the vegetation in the restored wetlands had similar plant biomass and species richness as the reference site before this study began (Kellogg and Bridgham 2002), and the vegetation arose entirely from dispersal, it is likely that sufficient numbers and species of fungal propagules such as spores and root fragments were also transported readily to the site via wind or animal vectors. A detailed analysis of the fungal species present would be necessary to determine whether the AM fungal community, not just the overall level of colonization, was similar in both the restored and reference sites and whether site amendments with AM fungi may be beneficial. For example, in terrestrial systems, obligate mycorrhizal species such as big blue stem (*Andropogon gerardii* Vitman) are excluded from areas where the appropriate fungal symbionts are not present (Hetrick *et al.* 1988), and thus, sites can be amended with mycorrhizal fungi to speed vegetative recovery. Similarly, it may be necessary to consider site amendments with AM fungal propagules during wetland restoration if arbuscular mycorrhizae prove to play an important functional role in wetland plant succession (Cooke and Lefore 1990, Turner *et al.* 2000).

Understanding the patterns of mycorrhizal colonization at various spatial and temporal scales, as in our study, is a necessary first step in understanding the role of AM fungi in wetland plant communities and ecosystem function. While our results and those of others highlight our lack of understanding of the patterns of mycorrhizal colonization in wetlands, we now have increasing evidence that mycorrhizal associations form in a range of wetland species, habitats, and soil conditions. Thus, it is important to consider AM fungi as a potentially important component of wetland ecosystems influencing plant community structure and ecosystem processes such as nutrient cycling, and with the potential to affect wetland restoration activities.

#### ACKNOWLEDGMENTS

We extend our gratitude to the many people who made this project possible, including Iris Charvat for introducing C.R.B. to the world of mycorrhizae, Shannon Torrence and Brad Herrick for field and laboratory

assistance and Barbara Hellenenthal for her enthusiastic help in plant identification. Members of the Lamberti and Bridgham labs and Dr. Paul Wetzel offered numerous comments that improved this manuscript. We also thank Tim Cordell and the staff of Potato Creek State Park, and Monica Paidle who allowed us access to the sites. This research was supported by a Claire Booth Luce memorial fellowship from the University of Notre Dame to C.R.B. and U.S. Geological Survey Grant No. 1434-HQ-96-GR-02669 through Purdue University.

#### LITERATURE CITED

- Abbott, L. K. and A. D. Robson. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment* 35:121–150.
- Allen, M. F. 1991. *The Ecology of Mycorrhizae*. Cambridge University Press, New York, NY, USA.
- Allen, M. F. 1996. *The Ecology of Arbuscular Mycorrhizas: a look back into the 20th century and a peek into the 21st*. *Mycological Research* 100:769–782.
- Allen, E. B. and M. F. Allen. 1990. The mediation of competition by mycorrhizae in successional and patchy environments. p. 367–389. *In* J. B. Grace and D. Tilman (eds.) *Perspectives on Plant Competition*. Academic Press, Inc., San Diego, CA, USA.
- Aziz, T., D. M. Sylvia, and R. F. Doren. 1995. Activity and species composition of arbuscular mycorrhizal fungi following soil removal. *Ecological Applications* 5:776–784.
- Beck-Nielsen, D. and T. Vindaek Madsen. 2001. Occurrence of vesicular-arbuscular mycorrhiza in aquatic macrophytes from lakes and streams. *Aquatic Botany* 71:141–148.
- Brown, A. M. and C. Bledsoe. 1996. Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. *Journal of Ecology* 84:703–715.
- Brundrett, M. 1991. Mycorrhizas in Natural Ecosystems. p. 171–277. *In* M. Begon, A. H. Fitter, and A. Macfadyen (eds.) *Advances in Ecological Research*, Volume 21. Academic Press, London, UK.
- Cooke, J. C. and M. W. Lefor. 1990. Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of coastal salt marsh in Clinton, Connecticut, USA. *Environmental Management* 14:131–137.
- Cooke, J. C. and M. W. Lefor. 1998. The mycorrhizal status of selected plant species from Connecticut wetlands and transition zones. *Restoration Ecology* 6:214–222.
- Cornwell, W. K., B. L. Bedford, and C. T. Chapin. 2001. Occurrence of arbuscular mycorrhizal fungi in a phosphorus-poor wetland and mycorrhizal response to phosphorus fertilization. *American Journal of Botany* 88:1824–1829.
- Francis, R. and D. J. Read. 1994. The contributions of mycorrhizal fungus to the determination of plant community structure. *Plant and Soil* 159:11–25.
- Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular infection in roots. *New Phytologist* 84:489–500.
- Harley, J. L. 1991. Introduction: the state of the art. p. 1–23. *In* J. R. Norris, D. J. Read, and A. K. Varma (eds.) *Methods in Microbiology*, Volume 23. Academic Press, London, UK.
- Hartnett, D. and G. Wilson. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* 80: 1187–1195.
- Hetrick, B. A. D., K. Gershefske, and G. W. T. Wilson. 1988. Mycorrhizal dependence and growth of warm-season and cool-season tallgrass prairie plants. *Canadian Journal of Botany* 66:1376–1380.
- Hetrick, B. A. D., D. C. Hartnett, G. W. T. Wilson, and D. J. Gibson. 1994. Effects of mycorrhizae, phosphorous availability, and plant

- density on yield relationships among competing tallgrass prairie grasses. *Canadian Journal of Botany* 72:168–176.
- Jackson, R. M. and P. A. Mason. 1984. *Mycorrhiza*. Edward Arnold Ltd., London, UK.
- Kellogg, C. H. and S. D. Bridgham. 2002. Colonization during early succession of restored freshwater marshes. *Canadian Journal of Botany* 80:176–185.
- Khan, A. G. 1974. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and on endogone spores in adjacent soils. *Journal of General Microbiology* 81:7–14.
- Kormanik, P. P. and A. C. McGraw. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. p. 37–45. *In* N. C. Schenck (ed.) *Methods and Principles of Mycorrhizal Research*. The American Phytopathological Society, St. Paul, MN, USA.
- Koske, R. E. and J. N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* 92:486–505.
- McGill, W. B. and C. T. Figueiredo. 1993. Total nitrogen. p. 201–212. *In* M.R. Carter, (ed.) *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, FL, USA.
- McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115:495–501.
- Miller, R. M., C. I. Smith, J. D. Jastrow, and J. D. Bever. 1999. Mycorrhizal status of the genus *Carex* (Cyperaceae). *American Journal of Botany* 86:547–553.
- Miller, S. P. 2000. Arbuscular mycorrhizal colonization of semi-aquatic grasses along a wide hydrologic gradient. *New Phytologist* 145:145–155.
- Miller, S. P. and R. R. Sharitz. 2000. Manipulation of flooding and arbuscular mycorrhiza formation influences growth and nutrition of two semiaquatic grass species. *Functional Ecology* 14:739–748.
- O'Halloran, I. P. 1993. Total and organic phosphorus. p. 213–230. *In* M.R. Carter (ed.) *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, FL, USA.
- Ozinga, W. A., J. van Andel, and M. P. McDonnell-Alexander. 1997. Nutritional soil heterogeneity and mycorrhiza as determinants of plant species diversity. *Acta Botanica Neerlandica* 46: 237–254.
- Powell, C. L. 1975. Rushes and sedges are non-mycotrophic. *Plant and Soil* 42:481–484.
- Read, D. J., H. K. Koucheki, and J. Hodgson. 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems. *New Phytologist* 77:641–653.
- Reeves, F. B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. *American Journal of Botany* 66:6–13.
- Rickerl, D. H., F. O. Sancho and S. Ananth. 1994. Vesicular-arbuscular endomycorrhizal colonization of wetland plants. *Journal of Environmental Quality* 23:913–916.
- Sharma, S., M. Madan, and P. Vasudevan. 1997. Biology and applications of mycorrhizal fungi. *Microbiologia Sem* 13:427–436.
- Sheldrick, B. H. and C. Wang. 1993. Particle size distribution. p. 499–512. *In* M. R. Carter (ed.) *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, FL, USA.
- Stenlund, D. L. and I. D. Charvat. 1994. Vesicular arbuscular mycorrhizae in floating wetland mat communities dominated by *Typha*. *Mycorrhiza* 4:131–137.
- Stevens, K. J. and R. L. Peterson. 1996. The effect of a water gradient on the vesicular-arbuscular mycorrhizal status of *Lythrum salicaria* L. (purple loosestrife). *Mycorrhiza* 6:99–104.
- Tang, F., J. A. White, and I. Charvat. 2001. The effect of phosphorus availability on arbuscular mycorrhizal colonization of *Typha angustifolia*. *Mycologia* 93:1042–1047.
- Tiessen, H. and J. O. Moir. 1993. Total and organic carbon. p. 187–200. *In* M.R. Carter (ed.) *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, FL, USA.
- Turner, S. D. and C. F. Friese. 1998. Plant-mycorrhizal community dynamics associated with a moisture gradient within a rehabilitated prairie fen. *Restoration Ecology* 6:44–51.
- Turner, S. D., J. P. Amon, R. M. Schneble, and C. F. Friese. 2000. Mycorrhizal fungi associated with plants in ground-water fed wetlands. *Wetlands* 20:200–204.
- Thormann, M. N., R. S. Currah, and S. E. Bayley. 1999. The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. *Wetlands* 19:438–450.
- van der Heijden, M. G. A., M. U. Klironomos, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72.
- Voss, E. W. 1972. Michigan Flora. Part I. Cranbrook Institute of Science and University of Michigan Herbarium, Bloomfield Hills, MI, USA. Bulletin 55.
- Voss, E. W. 1985. Michigan Flora. Part II. Cranbrook Institute of Science and University of Michigan Herbarium, Bloomfield Hills, MI, USA. Bulletin 55.
- Voss, E. W. 1996. Michigan Flora. Part III. Cranbrook Institute of Science and University of Michigan Herbarium, Bloomfield Hills, MI, USA. Bulletin 61.
- Wetzel, P. R. and A. G. van der Valk. 1996. Vesicular-arbuscular mycorrhizae in prairie pothole wetland vegetation in Iowa and North Dakota. *Canadian Journal of Botany* 74:883–890.
- White, J. A. and I. Charvat. 1999. The mycorrhizal status of an emergent aquatic, *Lythrum salicaria* L., at different levels of phosphorus availability. *Mycorrhiza* 9:191–197.
- Wigand, C. and J. C. Stevenson, 1994. The presence and possible ecological significance of mycorrhizae of the submersed macrophyte, *Vallisneria americana*. *Estuaries* 17:206–215.

Manuscript received 5 August 2002; revisions received 21 May 2003; accepted 18 August 2003.