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ABSTRACT

Many rhizosphere microorganisms enhance nutrient uptake and plant growth, but their effectiveness can vary with host species and with genotype within species. This study evaluated the effectiveness of rhizosphere microflora indigenous to the rhizosphere of switchgrass (*Panicum virgatum* L.) for enhancing seedling yield and nutrient uptake. Switchgrass roots and rhizosphere soil were collected from native prairies and seeded stands in Nebraska, Kansas, Iowa, Missouri, Virginia, and North Carolina. Seedlings of four switchgrass cultivars were inoculated with root fragments and rhizosphere soil from each collection, fertilized with a nutrient solution, and grown in steamed sand for 12 wk in a greenhouse. Seedlings inoculated with rhizosphere microflora produced up to 15-fold greater shoot and root yields, and recovered up to 6-fold more N and 36-fold more P than seedlings inoculated with rhizosphere bacteria only. These responses were consistent for all four switchgrass cultivars and were probably due to arbuscular mycorrhizal fungi. Switchgrass rhizosphere populations were highly variable in their ability to recover N and P and stimulate seedling shoot and root yields. Seedlings inoculated with rhizosphere populations from seeded switchgrass stands averaged 1.5-fold greater shoot and root yields than seedlings inoculated with rhizosphere populations from native prairies. Rhizosphere populations that stimulated the greatest N uptake differed from populations that resulted in the greatest P uptake. Highly effective microbial populations appear to develop in the rhizosphere of seeded switchgrass stands.

O VER THE PAST 20 YEARS, there has been a major increase in use of switchgrass (*Panicum virgatum* L.) for hay, summer grazing, soil conservation, and wildlife habitat (Moser and Vogel, 1995). Currently, there is interest in switchgrass as an energy crop (Moser and Vogel, 1995) because of its perennial nature, wide geographic distribution, high biomass yields, and ability to grow well in strongly acid soils with high exchangeable Al^{3+} concentrations (Balasko et al., 1984; Jung et al., 1988).

The profitability of switchgrass as a forage or biomass crop may be enhanced if yields can be increased with a concomitant decrease in chemical inputs, particularly N and P fertilizers. Research with other forage and grain crops indicates that rhizosphere microflora, particularly arbuscular mycorrhizal fungi (AMF), can enhance N and P uptake (Abbott and Robson, 1984; Brejda et al., 1993; George et al., 1995), water uptake and drought tolerance (Safir et al., 1971; Allen, 1982; Sylvia and Williams, 1992), and protect against or enhance plant tolerance to rhizosphere pathogens (Paulitz and Linderman, 1991; Linderman, 1994). However, not all AMF populations and isolates are equally effective (Abbott and Robson, 1982; Medina et al., 1988; Hung et al., 1990), and some species can be parasitic on their host (Modjo and Hendrix, 1986; Hendrix et al., 1992; Johnson et al., 1992). The variation in effectiveness among different AMF indicate that, if AMF are to enhance nutrient uptake and yield of switchgrass, selection of more effective AMF is needed (Daft, 1992).

Other rhizosphere microorganisms influence the establishment and activity of AMF (Paulitz and Linderman, 1991; Linderman, 1992). Evaluation of plant growth and nutrient uptake responses to inoculation with pure mycorrhizal cultures in sterile soil may significantly overestimate AMF effects, because other soil microorganisms are not considered (Hetrick et al., 1988; Koide and Li, 1989). Inoculum collected from switchgrass roots and rhizosphere soil in the field offers an advantage over pure cultures because it contains microbial populations already adapted to switchgrass under natural conditions. This suggests that, for some agriculturally based studies, rhizosphere inoculum may be most appropriate (Smith and Smith, 1981). However, there is no information on the effectiveness of different microbial populations indigenous to the rhizosphere of switchgrass. Our objective was to evaluate the effectiveness of different microflora populations indigenous to the rhizosphere of switchgrass plants for enhancing switchgrass seedling yield and nutrient uptake.

MATERIALS AND METHODS

Between 9 and 22 Oct. 1993, blocks (20 by 20 by 30 cm deep) of switchgrass roots and rhizosphere soil were collected directly beneath plant crowns in native prairies and seeded stands at 13 sites in six central and eastern states of the USA (Table 1). The 13 sites were chosen because they represent a diverse range in soil physical and chemical properties (Table 2). At each site, three plants located in the middle two-thirds of the stand were randomly selected for sampling. The blocks of soil containing switchgrass crown and root material, as collected in the field, were stored in a refrigerator at 4°C until they were processed.

Inoculum from each collection was prepared by compositing subsamples from the three plants sampled at each site and sifting the rhizosphere soil through a 2-mm sieve to remove rocks and large litter fragments. All adhering root material was removed and the sieves were cleaned in distilled water and

Abbreviations: AMF, arbuscular mycorrhizal fungi.

Table 1. State, county, switchgrass cultivar, and soil from which different rhizosphere populations were collected for evaluating their effectiveness with switchgrass.

Location	County	Switchgrass cultivar	Soil
Iowa			
Doolittle Prairie	Story	native	Kossuth silty clay loam (Typic Endoaquolls)
Ames	Story	Cave-in-Rock	Sparta loamy fine sand (Entic Hapludolls)
Kansas			
Flinthills prairie	Pottawatomie	native	Pawnee clay loam (Aquertic Argiudolls)
Manhattan	Pottawatomie	Blackwell	Wymore silt loam (Aquertic Argiudolls)
Missouri			
Kickapoo Prairie	Lawrence	native	Keeno cherty silt loam (Mollic Fragiudalfs)
Elsberry	Lincoln	Cave-in-Rock	Kampville silt loam (Typic Endoaqualfs)
Clifton Hill	Randolph	Blackwell	Piopolis silty clay loam (Typic Fluvaquents)
Nebraska			
Mead	Saunders	Pathfinder	Sharpsburg silty clay loam (Typic Argiudolls)
Nine-Mile Prairie	Lancaster	native	Pawnee–Burchard clay loam (Aquertic–Typic Argiudolls)
Sandhills prairie	Grant	native	Valentine fine sand (Typic Ustipsamments)
North Carolina			
Raleigh	Wake	Cave-in-Rock	Cecil sandy loam (Typic Kanhapludults)
Virginia			
Blacksburg	Montgomery	Alamo	Shottower loam (Typic Paleudults)
Blacksburg	Montgomery	Cave-in-Rock	Shottower loam (Typic Paleudults)

allowed to dry between samples to avoid cross-contamination. Additional root material was clipped free from the rhizomes and crown, cut into 2-cm pieces, and combined with the soil sievings.

Seed of four switchgrass cultivars were surface sterilized by soaking in a 0.07 M solution of NaOCl for 15 min, rinsed in distilled water, then germinated in vermiculite. The four cultivars evaluated were two lowland ecotypes, 'Alamo' and 'Kanlow', and two upland ecotypes, 'Cave-in-Rock' and 'Trailblazer'. Pots (1.9 L) containing steamed sand were inoculated with 55 cm³ of soil and roots from each collection. The inoculum was placed in a 5-mm-thick band 2.5 cm below the soil surface, and three uniform seedlings of each cultivar were transplanted at 3 wk of age into each pot. All pots received 25 mL of a composite bacterial filtrate to equalize the background microflora (i.e., microflora apart from AMF and associated rhizosphere fungi). The filtrate was added because AMF live

and function in the rhizosphere in association with other microorganisms, and their inclusion is needed to accurately assess the effect of AMF on plant growth and nutrient uptake. The filtrate was prepared by mixing together 55 cm³ of inoculant from each collection site, adding 6 L of water, and filtering the resultant slurry through a 20-μm sieve with a peristaltic pump (Linderman and Hendrix, 1982). Switchgrass seedlings receiving only the composite bacterial filtrate were included as a control treatment. Plants were grown in a greenhouse supplemented with sodium-vapor lamps (800 μmol photon m⁻² s⁻¹) to provide a 14-h photoperiod. The greenhouse temperature was maintained between 16 and 32°C. All pots were fertilized with 1 L of Clark's nutrient solution (Clark, 1982), which was modified to provide the equivalent of 4 mg P L⁻¹ and 70 mg N L⁻¹. The nutrient solution was added in 100-mL aliquots at weekly intervals for 10 wk.

Switchgrass shoots and roots were harvested after 12 wk,

Table 2. Physical and chemical properties of soils from which different rhizosphere populations were collected for evaluating their effectiveness with switchgrass. All soil test procedures follow Dahnke (1988).

Location	Particle size†			pH‡	Organic C§	Total N¶	Avail. P#	CEC††	Exchangeable††		
	Sand	Silt	Clay						Ca	Mg	K
	——— % ———				g kg⁻¹	—— mg kg⁻¹ ——			——— cmol_c kg⁻¹ ———		
Iowa											
Doolittle Prairie	27	45	28	6.3	4.4	7.6	3	51	30.1	7.7	0.4
Ames	85	8	7	6.7	1.4	2.5	147	11	6.4	1.4	0.6
Kansas											
Flinthills Prairie	15	71	14	6.5	4.4	6.9	13	35	19.7	4.1	1.2
Manhattan	8	55	37	6.9	2.5	3.7	35	45	24.2	6.2	1.2
Missouri											
Kickapoo Prairie	11	73	16	5.6	3.3	5.3	4	23	9.8	0.8	0.2
Elsberry	6	78	16	7.1	1.3	2.1	49	15	14.3	1.3	0.4
Clifton Hill	8	80	12	7.0	2.0	3.4	54	32	21.7	3.3	0.4
Nebraska											
Mead	10	64	26	6.0	3.4	5.4	58	28	13.0	3.8	2.8
Nine-Mile Prairie	29	51	20	6.0	2.8	4.6	9	25	11.8	3.0	1.0
Sandhills Prairie	87	5	8	6.4	0.6	0.7	8	5	2.4	0.5	0.3
North Carolina											
Raleigh	69	16	16	6.8	1.6	2.2	166	8	4.9	1.6	0.4
Virginia											
Blacksburg (Alamo)	36	47	17	5.1	1.5	2.7	92	10	1.0	0.6	0.5
Blacksburg (CIR‡‡)	45	39	16	5.5	1.4	2.4	86	11	1.4	0.6	0.3

† Bouyoucos hydrometer method.
‡ 1:1 soil:water.
§ LECO Cr12 carbon analyzer.
¶ LECO FP428 nitrogen analyzer.
# Bray and Kurtz No. 1 with ascorbic acid modification.
†† 1 M NH₄OAc extract at pH 7.0 and determined using atomic absorption.
‡‡ CIR, Cave-in-Rock.

Table 3. Analysis of variance and mean squares for four switchgrass cultivars inoculated with 13 different rhizosphere populations, or a bacterial filtrate control in two separate experiments.[†]

Source of variation	df	Shoot wt.	Root wt.	N uptake	P uptake	Colonization
Experiment (Exp)	1	3.4**	32.7**	13 470**	32.6**	[‡]
Inoculant (Inoc)	13	33.7**	23.3**	4 370**	68.3**	2 887**
Switchgrass cultivar[§]	3	19.1*	1.6	975**	2.6	402*
lowland vs. upland	(1)	56.6**	1.7	2 888**	0.7	437
Alamo vs. Kanlow	(1)	0.0	1.7	3	6.7	8.6
CIR vs. Trailblazer	(1)	0.7	1.5	31	0.5	761*
Exp × Inoc	13	2.7**	2.5**	282**	6.4**	
Exp × Cultivar	3	1.5	0.9	20	6.8**	
Inoc × Cultivar	39	1.4	1.2	190	3.2	231
Exp × Inoc × Cultivar	39	0.7	0.6	113	2.3	
Error	336	0.8	0.6	108	1.7	159

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† Experiments were analyzed as a random effect, and inoculants and switchgrass cultivars were fixed effects.
‡ Percentage colonization was determined in the second experiment only.
§ Alamo and Kanlow are lowland ecotypes; Cave-in-Rock (CIR) and Trailblazer are upland ecotypes.

dried separately at 60°C for 48 h in a forced-air oven, and weighed. The shoot and root materials were ground separately in a Wiley mill to pass a 1-mm screen and analyzed for total N and P concentrations. Total N was determined using the Kjeldahl procedure, and P by X-ray fluorescence (Knudsen et al., 1981). Nitrogen and P uptake was calculated by multiplying shoot and root N and P concentrations by shoot and root dry weights. The experiment was repeated with a second field collection of switchgrass roots and rhizosphere soil, collected between 26 April and 15 May 1994 from the same sites sampled previously, and 18-d-old seedlings using the same procedures described above. In the second experiment, a root subsample was taken directly from the zone of inoculation, cleared in KOH (25 g L^{-1}), and stained with trypan blue (0.5 g L^{-1}) in acid glycerol using the procedure of Koske and Gemma (1989). Percentage mycorrhizal colonization was determined by examining 100 root intercepts at 25× magnification using the grid–line intersect method of Giovannetti and Mosse (1980). The first experiment was planted on 26 January and harvested on 20 April; the second experiment was planted on 23 May and harvested on 15 August.

Both experiments were replicated four times and analyzed

as a factorial arrangement of 2 experiments, 4 switchgrass cultivars, and 14 microbial treatments (including the bacterial filtrate control). The analysis of variance involved a mixed model in which the experiments were treated as random effects and the switchgrass cultivars and microbial treatments as fixed effects. Microbial treatment means were compared using Fisher's protected LSD test at the 0.05 significance level. Switchgrass cultivar differences were analyzed using preplanned orthogonal contrasts to compare (i) the upland vs. lowland ecotypes and (ii) the two cultivars within each ecotype.

RESULTS

Shoot and Root Yields

In both experiments, inoculation of switchgrass seedlings with different rhizosphere populations increased shoot and root weights up to 16-fold compared with seedlings receiving only the bacterial filtrate control. Although there was a significant ($P < 0.01$) experiment ×

Table 4. Mean values (pooled across switchgrass cultivars) of shoot weights, root weights, N uptake, and P uptake for each of the inoculants in the two experiments. Percentage colonization was measured in the second experiment only.

Inoculum collection site	1st Exp.				2nd Exp.				
	Shoot wt.	Root wt.	N uptake	P uptake	Shoot wt.	Root wt.	N uptake	P uptake	Colonization
	— g pot^{-1} —		— mg pot^{-1} —		— g pot^{-1} —		— mg pot^{-1} —		%
Iowa									
Doolittle	1.9	1.2	48	2.0	2.1	1.6	34	2.4	1
Ames	3.0	2.6	64	4.0	3.6	3.3	47	5.1	3
Kansas									
Flinthills	2.5	1.6	50	2.8	2.6	2.2	46	3.2	13
Manhattan	3.5	2.6	63	4.2	4.3	3.7	52	5.4	33
Missouri									
Kickapoo	1.5	1.2	44	1.9	1.9	1.7	33	2.2	6
Elsberry	3.6	2.6	57	3.7	2.8	2.7	47	3.8	4
Clifton Hill	4.0	3.0	57	5.6	4.3	3.9	53	7.8	44
Nebraska									
Mead	3.2	2.2	64	4.0	4.6	3.9	55	4.8	18
Nine-Mile Prairie	2.7	2.5	65	3.1	2.0	1.9	39	2.7	1
Sandhills Prairie	3.0	2.1	53	3.4	3.3	2.8	46	4.1	22
North Carolina									
Raleigh	3.1	2.6	59	3.5	3.7	3.3	48	5.6	17
Virginia									
Blacksburg (Alamo)	2.7	2.1	48	3.6	2.3	2.1	40	2.7	3
Blacksburg (CIR†)	3.0	1.9	51	3.8	2.8	2.6	43	3.6	3
Bacterial filtrate	0.3	0.2	11	0.3	0.3	0.3	9	0.2	0
SE	0.4	0.3	5	0.5	0.5	0.4	5	0.8	6
LSD (0.05)	1.0	0.9	15	1.5	1.4	1.2	13	2.1	17

† CIR, Cave-in-Rock.

Table 5. Mean values (pooled across inoculants) of shoot weights, root weights, N uptake, and P uptake for each of the switchgrass cultivars in the two experiments.

	1st Exp.				2nd Exp.				
Cultivar	Shoot wt.	Root wt.	N uptake	P uptake	Shoot wt.	Root wt.	N uptake	P uptake	Colonization
	—— g pot^{-1} ——		—— mg pot^{-1} ——		—— g pot^{-1} ——		—— mg pot^{-1} ——		%
Alamo	3.0	2.1	56	3.3	3.3	2.8	44	4.2	10.8
Kanlow	3.2	2.0	56	3.4	3.2	2.5	45	3.3	10.3
Cave-in-Rock	2.3	2.0	51	3.3	2.7	2.7	40	4.1	15.9
Trailblazer	2.4	2.0	50	3.5	2.4	2.3	39	3.7	10.7
SE	0.4	0.3	5	0.5	0.5	0.4	5	0.8	6.3

microbial inoculant interaction for both shoot and root weights (Table 3), the magnitude of the interaction was small and resulted from small changes in the response observed with some of the microbial inoculants.

Consistent patterns were evident in both experiments with respect to the effects of the different rhizosphere populations on shoot and root weights. Seedlings with the greatest shoot and root weights were inoculated with rhizosphere microflora from seeded switchgrass stands near Clifton Hill, MO, Manhattan, KS, and Mead, NE (Table 4). In contrast, seedlings with the lowest shoot and root weights were inoculated with rhizosphere microflora from native switchgrass plants at Kickapoo Prairie in Missouri and Doolittle Prairie in Iowa (Table 4).

Switchgrass shoot weights, but not root weights, were greater with the two lowland ecotypes, Alamo and Kanlow, than with the two upland ecotypes, Cave-in-Rock and Trailblazer (Table 5); however, there was no significant difference in shoot weights between the two cultivars within each ecotype. Interactions between switchgrass cultivars and microbial inoculants or experiments were not significant for shoot and root weights (Table 3).

Shoot and root weights were positively correlated with the pH of the soil used as inoculum (Table 6). Root weights, but not shoot weights, were also positively correlated with available P, and negatively correlated with organic C and total N concentrations in the soil used as inoculum.

Nitrogen and Phosphorus Uptake

In both experiments, inoculation of switchgrass seedlings with different rhizosphere populations increased N uptake as much as 6-fold, and P uptake up to 37-fold compared with seedlings receiving only the bacterial filtrate control. As with shoot and root weights, there

was a significant ($P < 0.01$) experiment × microbial inoculant interaction for N and P uptake (Table 3), but the magnitude of the interaction was small and resulted from small changes in the amount of N and P taken by seedlings inoculated with some of the microbial populations.

Consistent patterns were evident in both experiments with respect to the effects of the different rhizosphere populations on N and P uptake. The rhizosphere microflora that produced high N and P uptake were collected from seeded stands near Mead, NE, and Manhattan, KS (Table 4). Seedlings inoculated with rhizosphere microflora from seeded stands at Clifton Hill, MO, and Ames, IA, also had high P uptake, but not high N uptake. In contrast, seedlings with the lowest N and P uptake were inoculated with rhizosphere microflora from native switchgrass plants at Kickapoo Prairie in Missouri, Doolittle Prairie in Iowa, Nine-Mile Prairie in Nebraska, and a seeded stand of Alamo near Blacksburg, VA (Table 4).

Uptake of N, but not of P, was significantly greater with the two lowland ecotypes, Alamo and Kanlow, than with the two upland ecotypes, Cave-in-Rock and Trailblazer (Tables 3 and 5). However, there was no significant difference in N uptake between the two cultivars within each ecotype. Interactions between switchgrass cultivars and microbial inoculants or experiments were not significant for N and P uptake (Table 3).

Nitrogen and P uptake were positively correlated with the pH of the soil used as inoculum (Table 6). In addition, N uptake, was positively correlated with exchangeable K in the soil used as inoculum.

Percentage Colonization

Mycorrhizal colonization of switchgrass seedlings varied significantly between the different rhizosphere populations (Table 3). Percentage colonization was signifi-

Table 6. Correlation (r) between shoot weight, root weight, N uptake, and P uptake (pooled across switchgrass cultivars) with soil properties at the 13 inoculum collection sites.

Soil property	Shoot wt.	Root wt.	N uptake	P uptake	Colonization
Sand	0.01	0.08	0.04	0.04	−0.19
Silt	−0.03	−0.08	−0.08	−0.01	0.18
Clay	0.07	−0.01	0.13	−0.11	0.12
pH	0.58†	0.58†	0.57†	0.57†	0.52†
Organic C	−0.39	−0.48†	−0.26	−0.42	−0.11
Total N	−0.43	−0.51†	−0.31	−0.44	−0.16
Avail. P	0.39	0.50†	0.37	0.44	−0.05
CEC	−0.05	−0.15	−0.02	−0.07	0.25
Exch. Ca	0.07	−0.03	0.07	0.06	0.32
Exch. Mg	0.06	−0.05	0.12	−0.01	0.23
Exch. K	0.38	0.30	0.57†	0.14	0.17

† Significant at $\alpha = 0.10$.

cantly ($P < 0.01$) correlated with shoot weight ($r = 0.77$), root weight ($r = 0.74$), N uptake ($r = 0.70$), and P uptake ($r = 0.82$). No mycorrhizal colonization was detected in switchgrass seedlings inoculated with only the bacterial filtrate in either experiment.

Mycorrhizal colonization was significantly greater with Cave-in-Rock switchgrass than with the other three cultivars (Table 5). The switchgrass cultivar × microbial population interaction was not significant. Mycorrhizal colonization was significantly correlated with the pH of the soil used as inoculum (Table 6); percentage colonization was not significantly correlated with any other property of the inoculant soil.

DISCUSSION

Several groups of microorganisms may be responsible for the large increases in shoot and root weights and in N and P uptake following inoculation of seedlings with rhizosphere microflora from established switchgrass stands. These groups include plant growth promoting rhizosphere bacteria, AMF, and nonmycorrhizal fungi such as saprophytes or pathogens. Studies using an inoculation method similar to our method have shown that rhizosphere bacteria can have a positive (Murdoch et al., 1967), negative (Wilson et al., 1988; Hetrick et al., 1988; Hetrick et al., 1990; Koide and Li, 1989), or no effect (Manjunath and Bagyaraj, 1981) on plant growth. However, enhanced plant growth resulting from inoculation with rhizosphere bacteria alone was consistently and significantly smaller than the effect of AMF on plant growth. The poor growth of switchgrass seedlings inoculated with only the bacterial filtrate in this study, consistent with results from the above-mentioned studies, indicates that rhizosphere bacteria probably were not responsible for the large increases in nutrient uptake and shoot and root yields observed on this study.

Changes in N and P availability through organic matter mineralization or nutrient immobilization by saprophytic bacteria and fungi could have affected seedling growth. However, the small and generally nonsignificant correlations between shoot yields or nutrient uptake, and organic C, total N, and available P in the soil used as inoculum suggest that the amounts of N and P in these soils did not have a strong influence on seedling yield or nutrient uptake. In addition, spores or oomycetes of saprophytic or pathogenic fungi were not observed in or around the roots that were stained and scored for percentage AMF colonization. Rather, the high correlations between percentage colonization and shoot and root weights and N and P uptake observed in the second experiment suggest that AMF are responsible for the responses observed in this study.

The most effective rhizosphere populations for increasing shoot and root weights and N and P uptake came from seeded switchgrass stands; rhizosphere populations from native prairies were relatively less effective. The greater effectiveness of AMF populations associated with long-term seeded switchgrass stands is in contrast to several studies indicating that, under monocropping systems, less effective or even parasitic AMF populations can develop (Modjo and Hendrix, 1986; Hendrix et al., 1992; Johnson et al., 1992). However,

all of these studies involved annual crops, and there is limited information on changes in the species composition and effectiveness of AMF in the rhizosphere of perennial plants. Our results suggest that AMF and associated rhizosphere microflora that are highly effective in enhancing seedling growth and nutrient uptake develop in the rhizosphere of switchgrass monocultures. The effectiveness of different rhizosphere populations in improving growth and nutrient uptake of inoculated switchgrass seedlings under field conditions needs to be evaluated.

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