Influence of mycorrhizal fungi and fertilization on big bluestem seedling biomass

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Abstract

The relationship between fertilization of prairie soils and mycorrhizal symbiosis in big bluestem (*Andropogon gerardii* Vit.) was explored. In 10 steamed prairie soils of varied P level, inoculation with a mycorrhizal fungus resulted in a 7- to 70-fold increase in big bluestem seedling biomass, compared to noninoculated controls. Fertilization with N and K (25-0-25) significantly increased biomass of mycorrhizal seedlings but did not alter growth of nonmycorrhizal seedlings. In a second experiment which assessed the impact of N and P on seedling growth, in both steamed and nonsterile soil, P fertilization did not significantly increase plant biomass, while N fertilization did substantially increase biomass of mycorrhizal, but not nonmycorrhizal plants. Fertilization with N and P together produced the greatest biomass in both mycorrhizal and nonmycorrhizal plants. Apparently, in the range soils tested N is the most limiting nutrient, despite the low P availability exhibited by these soils. In the absence of mycorrhizae, however, P is most limiting and no response to N is observed unless sufficient P is also applied. These studies confirm an extremely important role for mycorrhizal fungi on big bluestem seedling growth.

Key Words: phosphorus, nitrogen, *Glomus etunicatum*, mycorrhizae, *Andropogon gerardii*

Tallgrass prairie soils of the Kansas Flint Hills contain little plant-available phosphorus as estimated by chemical soil tests (Halm et al. 1972). In spite of this, these prairie soils are productive and plants grown in these soils do not respond to phosphorus fertilization (Moser and Anderson 1964). Hall et al. (1984) suggested that nitrogen fertilization can raise N/P ratios in shoots, in effect lowering plant P status and increasing demand for P. In prairie soils, however, even following N fertilization which significantly increases plant growth (Moser and Anderson 1964; Rains et al. 1975; Woolfolk et al. 1975; Wallace 1981), there is no significant response to P fertilization (Mader 1956). Presumably adequate supplies of phosphorus are made available for plant growth by microbial mineralization of organic P (Coleman et al. 1983). Other mechanisms by which soil microorganisms contribute to the P nutrition of prairie plants have received less attention.

Vesicular-arbuscular mycorrhizal (VAM) fungi are abundant in grassland soils (Hetrick and Bloom 1983, Dickman et al. 1984, Stahl and Christensen 1982) and readily colonize roots of prairie grasses and forbs (Hetrick and Bloom 1983, Zajicek et al. 1986). Warm-season prairie grasses and forbs display a high degree of dependence on mycorrhizae and to not appear to survive without the symbiosis (Hetrick et al. 1988a). While these fungi can increase plant drought tolerance and resistance to soil-borne pathogens, their primary role in the ecosystem may be to aid plants in acquiring soil nutrients (Hayman 1983). Hyphae in soil are abundant, highly branched, and extend beyond the zone of depletion which surrounds plant roots (Tinker and Gildon 1982). These hyphae absorb nutrients from soil and translocate them into the plant cortex. Thus, more surface area for nutrient absorption is available, allowing 4 times faster uptake rates of phosphorus as compared with nonmycorrhizal roots (Sanders and Tinker 1973). Grassland plants may be connected to each other by hyphae of mycorrhizal fungi and nutrients may flow between plants via these hyphal bridges (Chiariello et al. 1982). More recently, Hetrick et al. (1988b) demonstrated that mycorrhizal plants display a more elongate root growth pattern, allowing a greater soil volume to be explored for nutrients. Thus, several mechanisms exist whereby mycorrhizal fungi may play a critical role in nutrient acquisition for prairie plants.

The purpose of this study was to explore the relationship between fertilization of prairie soils and mycorrhizal symbiosis in big bluestem (*Andropogon gerardii* Vit.) seedlings.

Materials and Methods

Experiment 1

Tallgrass prairie soil was collected from 10 sites on Konza Prairie Research Natural Area (KPRNA), near Manhattan, Kans. Sites 1, 2, 4, and 5 were Chase silty clay loams (fine, montmorillonitic, mesic Aguic Argiudoll), and sites 3, 6, 7, 8, 9 and 10 were silty and cherty clay loams of the Benfield-Florence complex. Benfield soils are fine, mixed, mesic Udic Argiustolls and Florence soils are clayey-skeletal, montmorrillonitic, mesic Udic, Arguistolls. Phosphorus content of the soils (Table 1) was determined using the Bray I method for extractable P (Olsen and Sommers 1982) by the Kansas State University Soil Testing Laboratory. One-half of each of the 10 soils was steam pasteurized for 2 hours at 100° C, while the other half remained nonsterile. Twenty-four replicate cylindrical pots (6 × 25 cm) were then filled with 475 g (dry weight) steamed or nonsterile soil from the 10 locations.

Big bluestem seedlings (0.01-0.04 g dry weight) which had been germinated and grown in vermiculite for 2 weeks were transplanted into each pot (one seedling/pot). One-half of the pots containing steamed or nonsterile soil received inoculum of *Glomus etunicatum* Becker and Gerd. collected from sudangrass (*Sorghum vulgare* var. *sudanense* [Piper] Hitch.) pot cultures maintained in a 15–25° C greenhouse. These pot cultures were initiated from *G. etunicatum* spores collected from KPRNA. This species was widespread and abundant in earlier surveys of KPRNA (Hetrick and Bloom 1983). The spores were recovered by wet sieving, decanting, and centrifuging on a 20-40-60% sucrose-density gradient (Daniels and Skipper 1982). Spores were pipeted (400 spores/plant) onto roots of each seedling at transplanting. The 12 pots of each treatment were then subdivided into 2 groups of 6 pots. Six of these pots received fertilization every other week with 0.063 g Peter's No-Phos (25-0-25) special fertilizer solution (Peter's Fertilizer Products, Fogelsville, Penn. 18051) delivered in 25 ml water, and the remaining 6 pots remained unamended. Therefore, approximately 35 ppm N and 29 ppm K were added to each pot every other week. The pots were arranged in a randomized com-

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Research was partially supported by the NSF LTER Program, grant number BSR-8514 327.

Contribution 89-16-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

Manuscript accepted 17 November 1988.

Table 1. The influence of mycorrhizal fungi on dry weight of big bluestem in 10 steamed or nonsterile range soils which were not fertilized or were amended with (25-0-25).

Soil/P level (ppm)	Total dry weight (g)[1]							
	Not fertilized				Fertilized[2]			
	Steamed soil		Nonsterile soil		Steamed soil		Nonsterile soil	
	Inoculated[3]	Noninoculated	Inoculated[3]	Noninoculated	Inoculated[3]	Noninoculated	Inoculated[3]	Noninoculated
1/1.5	1.20^{c}	0.02^{e}	0.64^{d}	0.68^{d}	7.44^{a}	0.02^{e}	5.14^{b}	5.29^{b}
2/1.5	1.51^{b}	0.02^{d}	0.58^{c}	0.69^{c}	4.20^{a}	0.03^{d}	1.45^{b}	1.51^{b}
3/3.0	1.52^{c}	0.03^{d}	1.31^{c}	1.12^{c}	3.76^{a}	0.04^{d}	2.62^{b}	2.47^{b}
4/14.0	7.58^{c}	0.93^{e}	3.64^{d}	3.37^{d}	15.24^{a}	0.34^{e}	11.61^{b}	11.84^{b}
5/2.5	2.96^{a}	0.04^{c}	0.94^{b}	0.84^{b}	3.14^{a}	0.03^{c}	2.90^{a}	2.42^{a}
6/2.5	1.42^{b}	0.02^{d}	0.44^{cd}	0.33^{cd}	3.44^{a}	0.05^{d}	1.08^{bc}	1.23^{b}
7/16.0	7.39^{cd}	6.30^{d}	7.36^{cd}	6.22^{d}	12.65^{a}	9.59^{bc}	10.56^{ab}	8.92^{bc}
8/6.0	3.53^{b}	0.45^{d}	1.18^{c}	1.27^{c}	5.66^{a}	0.13^{d}	3.53^{b}	3.55^{b}
9/3.5	1.55^{c}	0.03^{e}	0.42^{d}	0.52^{d}	2.77^{a}	0.02^{e}	2.17^{b}	1.79^{bc}
10/3.0	2.58^{b}	0.03^{d}	0.66^{cd}	0.80^{c}f	6.13^{a}	0.12^{d}	2.22^{b}	2.72^{b}

[1]Row means within each soil followed by the same letter are not significantly ($P = 0.05$) different as determined using Duncan's multiple-range test.
[2]Fertilized with (25-0-25).
[3]Inoculated with spores of *G. etunicatum*.

plete block design and maintained in a 15–25° C greenhouse.

Experiment 2

To further assess the influence of fertilization on big bluestem dry weight production, tallgrass prairie soil was collected from site 1 (1.5 ppm P). One-half of the soil was steam pasteurized. Pots (6 × 25 cm) were filled with 500 g (dry weight) steamed or nonsterile soil. One-half of the pots in each soil treatment were inoculated with *G. etunicatum* (400 spores/pot). These treatments were then subdivided into 7 groups of 24 pots, each group containing 6 replicate pots of each soil and inoculation treatment. The 24 pots of each group received 1 of the following fertilizer treatments: (1) no fertilizer; (2) low P (10 ppm applied at experiment setup) with K (30 ppm applied every other week); (3) high P (25 ppm P at setup) with K; (4) N (35 ppm N applied every other week) with K; (5) N with low P and K; (6) N with high P and K; (7) K alone. P, N and K were applied in 10 ml aliquots of KH_2PO_4, NH_4NO_3 and KCl solutions, respectively. To eliminate any pH effects, phosphorus was added as KH_2PO_4. Then, to ensure that potassium levels were similar in all treatments, potassium was added alone or in combination with N also. Each solution was applied to the soil surface and pots and were then watered to saturation. This study was repeated twice. The pots were arranged in a 15–25° C greenhouse in a completely randomized design.

Both studies were maintained in the greenhouse for 14 weeks, at which time plants were harvested and roots washed free of soil. Plants were dried at 90° C for 2 to 3 days, and root and shoot dry weights determined to the nearest mg. Shoot dry weights and root dry weights were each highly correlated with total plant dry weight ($R^2 > 0.85$ for Experiment 1 and $R^2 > 0.98$ for Experiment 2). Thus, for simplication of data presentation only total dry weights are presented for each experiment. Root/shoot ratios were also assessed but no clear trends were revealed and these data are, therefore, not shown. Subsamples of dried roots were stained in trypan blue (Phillips and Hayman 1970) and examined microscopically to assess percentage root colonization using a petri plate scored into 1-mm squares. Differences in total dry weight and percentage root colonization were subjected to analysis of variance ($P = 0.05$). Analyses of Experiments 1 and 2 revealed many significant interactions which precluded comparisons of main effects. Therefore, data from both experiments were subjected to Duncan's multiple-range test for mean separation.

Results

Experiment 1

When 10 soils frm Konza Prairie were compared, P levels ranged between 1.5 and 16 ppm. In all but one of the steamed soils which had not been fertilized, there was a significant benefit from mycorrhizal fungus inoculation (Table 1). Mycorrhizal plants had 7 to 70 times more biomass than nonmycorrhizal plants. Nonmycorrhizal plants were stunted and failed to grow appreciably, except in soils with the highest P levels (soils 4, 7, and 8). Growth of inoculated plants was also greatest in soils with higher P contents.

Growth of nonfertilized plants in nonsterile soil was similar whether or not they were inoculated, presumably because nonsterile soil contains sufficient indigenous inoculum of mycorrhizal fungi. The indigenous mycorrhizal fungus population may also be responsible for the significantly better growth of plants in nonsterile soil as compared with noninoculated plants in steamed soil. Growth in nonsterile soil, either inoculated or noninoculated, was (in all but 2 soils) significantly less than in steamed, inoculated soil.

In all 10 soils, inoculated plants in steamed soil fertilized with N and K had significantly more biomass than nonfertilized, inoculated plants. However, fertilization had no effect on noninoculated plants in steamed soil except in soil 7, the soil with the highest P content. Fertilized inoculated plants in steamed soil averaged 105 times greater growth than fertilized noninoculated plants in steamed soil as compared with nonfertilized inoculated plants in steamed soil, which averaged 46 times greater growth than nonmycorrhizal control plants in steamed soil.

Roots of inoculated big bluestem grown in nonfertilized, steamed soil were 23–65% colonized by *G. etunicatum* while roots of plants grown in nonsterile nonfertilized soil were 6–19% colonized (Table 2). Fertilization of inoculated big bluestem significantly increased root colonization levels in all 10 steamed soils, but fertilization had no effect on root colonization in either inoculated or noninoculated nonsterile soil.

Experiment 2

Generally, there was no significant growth response to either level of phosphorus fertilization in steamed or nonsterile soil whether or not plants were inoculated (Table 3). However, growth in noninoculated, steamed soil did improve with P fertilization ($P = 0.08$). The P levels used in this experiment may have been too low to elicit a significant growth response. In previous experiments in which N was not limiting, P fertilization significantly improved growth of nonmycorrhizal plants in steamed soil (Hetrick et al. 1988b). In contrast, as in Experiment 1, N fertilization significantly

Table 2. The influence of fertilization on mycorrhizal root colonization of big bluestem in steamed and nonsterile soil.

	Root colonization (%)[1]							
	Not fertilized				Fertilized[2]			
	Steamed soil		Nonsterile soil		Steamed soil		Nonsterile soil	
Soil	Inoculated[3]	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated
1	52.4^{b}	0^{d}	18.4^{c}	17.2^{c}	74.2^{a}	0^{d}	18.0^{c}	18.8^{c}
2	34.8^{b}	0^{f}	6.4^{e}	7.0^{de}	54.6^{a}	0^{f}	12.6^{cd}	17.2^{c}
3	47.2^{b}	0^{e}	10.4^{cd}	8.0^{d}	54.4^{a}	0^{e}	15.8^{c}	13.0^{cd}
4	60.6^{b}	0^{d}	19.2^{c}d	16.6^{c}	80.8^{a}	0^{d}	22.2^{c}	18.4^{c}
5	53.6^{b}	0^{d}	6.6^{c}	7.4^{c}	71.8^{a}	0^{d}	8.8^{c}	7.0^{c}
6	32.6^{b}	0^{e}	16.0^{cd}	11.8^{d}	47.8^{a}	0^{e}	20.2^{c}	12.4^{d}
7	23.2^{b}	0^{e}	15.0^{cd}	12.2^{d}	37.8^{a}	0^{e}	18.6^{bc}	16.0^{cd}
8	60.4^{b}	0^{d}	16.6^{c}	13.6^{c}	82.6^{a}	0^{d}	12.8^{c}	15.0^{c}
9	65.8^{b}	0^{d}	12.4^{c}	11.0^{c}	77.6^{a}	0^{d}	15.4^{c}	14.0^{c}
10	35.8^{b}	0^{e}	9.4^{cd}	7.8^{d}	76.8^{a}	0^{e}	14.6^{c}	7.8^{d}

[1]Row means within each soil followed by the same letter are not significantly different ($P = 0.05$) as determined using Duncan's multiple-range test.
[2]Fertilized biweekly with 0.063 g/pot Peter's No-Phos (25-0-25) special fertilizer solution.
[3]Inoculated with 400 spores *G. etunicatum*.

increased growth of inoculated plants in steamed or nonsterile soil but had no effect on noninoculated plants in steamed soil. Nitrogen fertilization was stimulatory to noninoculated plants in nonsterile soil, again presumably because these plants were colonized by indigenous mycorrhizal fungi.

Table 3. Effect of nitrogen and phosphorus fertilization on growth of mycorrhizal and nonmycorrhizal big bluestem in prairie soil.

	Total dry weight (g)[1]			
	Steamed soil		Nonsterile soil	
Fertilization treatment[2]	Inoculated[3]	Non-inoculated	Inoculated	Non-inoculated
No fertilizer	0.92^{fgh}	0.41^{i}	0.96^{fgh}	0.92^{fgh}
P_1 + K	1.13^{fg}	0.18^{i}	0.84^{fgh}	0.87^{fgh}
P_2 + K	1.10^{fg}	0.56^{ghi}	0.82^{fgh}	0.84^{fgh}
N + K	6.59^{c}	0.04^{i}	4.84^{e}	5.54^{d}
P_1 + N + K	7.70^{b}	0.35^{hi}	5.33^{de}	5.21^{de}
P_2 + N + K	8.47^{a}	1.35^{f}	7.43^{b}	7.10^{bc}
K alone	0.99^{fgh}	0.07^{i}	0.91^{fgh}	0.87^{fgh}

[1]Means for the entire experiment followed by the same letter are not significantly ($P = 0.05$) different as determined using Duncan's multiple-range test.
[2]P_1 = 10 ppm P at setup.
P_2 = 25 ppm at setup.
N = 35 ppm N every other week.
K = 30 ppm K every other week.
[3]Inoculated with 400 *G. etunicatum* spores.

When N and the lower level of P were added together, growth of inoculated plants in steamed soil exceeded that of plants which received only N. This P response was not shared by noninoculated plants except at the higher P level. It was only when the highest P level was applied with N that maximum growth was achieved for plants in all treatments.

Mycorrhizal root colonization was significantly reduced in inoculated plants which received the higher level of P fertilizer in steamed soil (Table 4). As in Experiment 1, N plus K fertilization stimulated root colonization of plants in steamed soil. However, even in these N fertilized plants, where P demand should be greatest, P fertilization was still detrimental to root colonization. In contrast, N plus P fertilization had no effect on root colonization in nonsterile soil, and the low level of root colonization observed in nonsterile soil was constant over all fertilizer treatments.

Table 4. Effect of nitrogen and phosphorus fertilization on mycorrhizal root colonization of big bluestem in prairie soil.

	Root colonization (%)[1]			
	Steamed soil		Nonsterile soil	
Fertilization treatment[2]	Inoculated[3]	Non-inoculated	Inoculated	Non-inoculated
No fertilizer	79.67^{bc}	0.0^{h}	10.00^{g}	9.33^{g}
P_1 + K	84.17^{b}	0.0^{h}	8.00^{g}	7.83^{g}
P_2 + K	57.50^{e}	0.0^{h}	8.67^{g}	9.17^{g}
N + K	91.00^{a}	0.0^{h}	9.83^{g}	9.17^{g}
P_1 + N + K	70.17^{d}	0.0^{h}	8.67^{g}	8.50^{g}
P_2 + N + K	52.20^{f}	0.0^{h}	10.00^{g}	8.67^{g}
K alone	78.2^{c}	0.0^{h}	9.83^{g}	9.20^{g}

[1]Means followed by the same letter are not significantly ($P = 0.05$) different as determined using Duncan's multiple-range test.
[2]P_1 = 10 ppm P at setup.
P_2 = 25 ppm P at setup.
N = 35 ppm N every other week.
K = 30 ppm K every other week.
[3]Inoculated with 400 *G. etunicatum* spores.

Discussion

In both experiments plants in nonsterile soil produced less biomass than inoculated plants in steamed soil, a phenomenon reported previously (Hetrick et al. 1986, 1988b, 1988c). This phenomenon has been attributed to microbial competition for nutrients, particularly phosphorus. In soils amended with phosphorus, (Hetrick et al. 1988b) and in soils with high P content (Kitt et al. 1988) microbial competition for phosphorus did not limit growth of big bluestem. In Experiment 1, soil 7 had the highest P content and no reduction of plant growth was observed in nonsterile soil compared to steamed soil, regardless of fertilization treatment. Hetrick et al. (1988b) have also observed that some prairie soils are so low in fertility that the growth limiting effects of microbial competition only occur following fertilization. This may explain why no suppression of plant growth was observed in nonsterile soil, as compared with growth in stemmed soil, in the second experiment until after fertilizer was applied. Since N plus K fertilization resulted in significant ($P = 0.05$) differences between inoculated plants in steamed and nonsterile soil, microbial competition for nitrogen may be as important as competition for P in

explaining the reduced growth of plants in nonsterile soil.

Mycorrhizal root colonization in nonsterile soil is also lower than in steamed soil (Table 4; also see Hetrick et al. 1988b, 1988c) and may or may not be related to the reduced suppression of growth response which occurs in nonsterile soil. Lower rates of root colonization in nonsterile soil could result from inhibition of spore germination (Wilson et al. 1989) or hyphal grazing by soil fauna (Fitter 1985). Indigenous mycorrhizal fungi may be more agressive colonizers of root tissue, displaying lower levels of root colonization, while effectively excluding the introduced fungus colonizing. Indeed, with at least 6 fungi (native and introduced) there was no relationship between colonization levels in steamed and nonsterile soil (Hetrick unpublished).

The lack of relationship between root colonization in steamed and nonsterile soil makes the stimulation of root colonization in steamed soil by nitrogen fertilization which was observed in both experiments difficult to interpret. Hall et al. (1984) suggested that N fertilization could increase P demand in plants. If N fertilization in effect creates a P deficiency in plants, then increased reliance on and increased root colonization by mycorrhizal fungi would be expected. However, microbial suppression of the mycorrhizal fungi may preclude any further colonization of roots despite the plants' increased need for P. Since N fertilization increased growth of mycorrhizal plants in both steamed and nonsterile soil, but increased root colonization only in steamed soil, the beneficial effect of N fertilization is probably a direct effect on plant growth, not a response mediated by the fungi. Thus the increase in root colonization in steamed soil following N fertilization may be a fungus response to improved host nutrition in the absence of microbial competition.

These studies confirm that nitrogen is the most limiting nutrient in range soils, despite the low available P status of these soils. However, in the absence of mycorrhizae, P is most limiting for seedlings and no N response is observed unless sufficient P is also applied. This implies an extremely important role for these fungi in big bluestem seedling growth. It is unclear from these studies to what extent mature plants rely on the symbiosis, but it is clear that mycorrhizal fungi make a significant contribution at least to seedling establishment. Seedling establishment may, therefore, depend upon range practices which encourage mycorrhizal symbiosis and the fungal symbiont's survival (e.g., avoid excessive P fertilization which may inhibit VAM fungi). Since N fertilization, while itself beneficial, does not appear to stimulate mycorrhizal root colonization in nonsterile soil, it is unlikely that this management practice can be used to improve mycorrhizal fungus root colonization. Had these experiments been conducted only in steamed soil the opposite conclusion might have been drawn. This underscores the importance of considering the soil microflora in rangeland studies. Further research will be necessary to assess whether seedling failures on reclaimed cropland are related to low populations of VAM fungi or phosphorus levels inhibitory to the symbiosis.

Literature Cited

Chiariello, N., J.C. Hickman, and H.A. Money. 1982. Endomycorrhizal role for interspecific transfer of phosphorus in a community of annual plants. Science 217:941-943.

Coleman, D.C., C.P.P. Reid, and C.V. Cole. 1983. Biological strategies of nutrient cycling in soil systems. *In:* A MacFadyen and E.D. Ford (eds.), Advances in ecological research, Vol. 13. Academic Press, New York.

Daniels, B.A., and H.D. Skipper. 1982. Methods for recovery and quantitative estimation of propagules from soil. p. 29-37. *In:* N.C. Schenck (ed.), Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Puall, Minn.

Dickman, L.A., A.E. Liberta, and R.C. Anderson. 1984. Ecological interaction of little bluestem and vesicular-arbuscular mycorrhizal fungi. Can. J. Bot. 62:2272-2277.

Fitter, A.H. 1985. Functioning of vesicular-arbuscular mycorrhizas under field conditions. New Phytol. 99:257-265.

Hall, I.R., P.D. Johnstone, and R. Dolby. 1984. Interactions between endomycorrhizas and soil nitrogen and phosphorus on the growth of ryegrass. New Phytol. 97:447-453.

Halm, B.J., J.W.B. Stewart, and R.H. Halstead. 1972. The phosphorus cycle in a native grassland ecosystem. p. 571-789. *In:* Isotopes and radiation in soil-plant relationships including forestry. Proc. of the Symposium, Vienna, 13–17 December 1974. STI/PuB/292, Vienna, IAEA.

Hayman, D.S. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can. J. Bot. 61:944-963.

Hetrick, B.A. Daniels, and J. Bloom. 1983. Vesicular-arbuscular mycorrhizal fungi associated with native tallgrass prairie and cultivated winter wheat. Can. J. Bot. 61:2140-2146.

Hetrick, B.A. Daniels, D. Gerschefske Kitt, and G. Thompson Wilson. 1986. The influence of phosphorous fertilization, drought, fungal species and soil microorganisms on mycorrhizal growth response in tallgrass prairie plants. Can. J. Bot. 64:1199-1203.

Hetrick, B.A. Daniels, D. Gerschkefske Kitt, and G.W.T. Wilson. 1988a. Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. Can. J. Bot. (In press).

Hetrick, B.A. Daniels, J.F. Leslie, G. Thompson Wilson, and D. Gerschefske Kitt. 1988b. Physical and topological assessment of VA-mycorrhizal fungus effects on root architecture of big bluestem. New Phytol. (In press).

Hetrick, B.A. Daniels, G. Thompson Wilson, D. Gerschefske, Kitt, and A.P. Schwab. 1988c. Soil microorganisms: effects on mycorrhizal contribution to growth of big bluestem in nonsterile soil. Soil Biol. & Biochem. (In press).

Kitt, D. Gerschefske, B.A. Daniels Hetrick, and G.W.T. Wilson. 1988. Relationship of soil fertility to suppression of mycorrhizal big bluestem growth response in nonsterile soil. New Phytol. (Accepted).

Mader, E.L. 1956. The influence of certain fertilizer treatments on the native vegetation of Kansas prairie. Ph.D. Diss., Univ. Nebraska, Lincoln.

Moser, L.E., and K.L. Anderson. 1964. Nitrogen and phosphorus fertilization of bluestem range. Trans. Kans. Acad. Sci. 67:613-616.

Olsen, S.R., and L.E. Sommers. 1982. Phosphorus. p. 403-430. *In:* A.L. Page, R.H. Miller, and D.R. Keeney, (eds.). Methods of soil analysis, Part 2. Chemical and microbiological properties, 2nd Ed. Amer. Soc. Agron., Inc., Soil Sci. Soc. Amer., Inc., Madison, Wis.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

Rains, J.R., C.E. Owensby, and K.E. Kemp. 1975. Effects of nitrogen fertilization, burning, and grazing on reserve constituents of big bluetem. J. Range Manage. 28:358-362.

Sanders, F.E., and P.B. Tinker. 1973. Phosphate flow in mycorrhizal roots. Pestic. Sci. 4:388-395.

Stahl, P.D., and M. Christensen. 1982. Mycorrhizal fungi associated with *Bouteloua* and *Agropyron* in Wyoming sagebrush grasslands. Mycologia 74:877-885.

Tinker, P.B., and A. Gildon. 1982. Mycorrhizal fungi and ion uptake (Chapter 2). p. 21-32. *In:* D.A. Robb and W.S. Pierpoint (eds.), Metals and micronutrients, uptake and utilization by plants. Academic Press, Phytochem. Soc. Eur. Symp. Ser. No. 21.

Wallace, L.L. 1981. Growth, morphology and gas exchange of mycorrhizal and nonmycorrhizal *(Panicum coloratum)* L., a C grass species under different clipping and fertilization regimes. Oecologia (Berlin) 49:272-278.

Wilson, G.W.T., B.A. Daniels Hetrick, and D. Gerschefske Kitt. 1989. Suppression of VA mycorrhizal fungus spore germination by nonsterile soil. Can. J. Bot. (In press).

Woolfolk, J.S., E.F. Smith, R.R. Schalles, B.E. Brent, L.H. Harbers, and C.E. Owensby. 1975. Effects of nitrogen fertilization and late-spring burning of bluestem range on diet and performance of steers. J. Range Manage. 28:190-193.

Zajicek, J.M., B.A. Daniels Hetrick, and C.E. Owensby. 1986. The influence of soil depth on mycorrhizal colonization of forbs in the tallgrass prairie. Mycologia 78:316-320.