Mycorrhizal influences on big bluestem rhizome regrowth and clipping tolerance

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Abstract

Mycorrhizal symbiosis is critical to growth of many warm-season prairie grass seedlings, but its effect on regrowth of rhizomes has not been determined. As forage species, the effect of grazing on the symbiosis is also important. When the impact of mycorrhizae on regrowth of *Andropogon gerardii* Vit. rhizomes was assessed, *A. gerardii* rhizomes collected from the field and grown with mycorrhizal inoculum produced larger plants than rhizomes grown in the absence of the symbiont. The effect of the symbiosis on clipping (simulated grazing) tolerance was quantified by growing *A. gerardii* in steamed or nonsterile prairie soil, with or without mycorrhizal fungus inoculation. Plants were clipped and a portion of the plants harvested at 6, 12, 18, 24, and 30 weeks after planting. As an additional control, Benomyl fungicide was applied to plants to inhibit the symbiosis. Mycorrhizal clipped plants were larger than nonmycorrhizal clipped plants, but the difference diminished with successive clippings. Mycorrhizal root colonization also decreased in response to repeated clipping. Maximum shoot and root biomass of mycorrhizal plants was produced at 12 and 18 weeks, respectively. Fungicide-treated plants did not grow appreciably after the first clipping. Thus, mycorrhizae improved clipping tolerance, but with repeated intensive clipping, significant changes in root/shoot ratio occurred and eventually mycorrhizal root colonization and growth benefit were lost.

Key Words: grazing, vesicular-arbuscular mycorrhizae, big bluestem, herbage yield

Warm-season, C4, range grasses provide summer forage in many areas of the Great Plains. These grasses live in a symbiotic association with vesicular-arbuscular mycorrhizal (VAM) fungi (Hetrick and Bloom 1983), and seedlings can not establish without them (Hetrick et al. 1989). Mycorrhizal symbiosis generally increases host-plant nutrient uptake, drought tolerance, and resistance to soil-borne pathogens and may also bind soil into stable aggregates which resist erosion (Hayman 1983, Mosse 1973, Menge 1986). In tallgrass prairie the symbiosis probably plays a particularly important role in acquisition of P, since available P is quite low in these soils and seedling growth is inhibited without the symbiosis or high levels of P fertilizer (Hetrick et al. 1989). The dominant warm-season grass, big bluestem (*Andropogon gerardii* Vit.), and other range grasses reproduce from rhizomes in which carbohydrate and mineral nutrient resources are stored throughout the winter and from which growth is initiated in spring. Therefore, the impact of VAM symbiosis on mature plants is, in many respects, more important than the role of these fungi in seedling establishment and must be clarified.

VAM symbiosis can significantly increase grazing tolerance (Wallace 1981). When clipping was used to simulate grazing, mycorrhizal plants maintained higher rates of photosynthesis and a more prostrate growth habit than nonmycorrhizal plants (Wallace 1981, Wallace and Svejcar 1987). The latter is important because it minimizes loss to herbivores (Wallace 1981). When grasses are heavily grazed, however, root colonization levels, mycorrhizal fungus spore numbers, and fungal biomass may decline and root/shoot ratios may increase (Bethlenfalvay and Dakessian 1984, Bethlenfalvay et al. 1985).

We evaluated the impact of VAM symbiosis on mature plants and examined the effect of these fungi on big bluestem plants exposed to repeated clipping.

Materials and Methods

To determine the contribution of mycorrhizal symbiosis to mature prairie plants, big bluestem rhizomes were collected from a monoculture plot near Manhattan, Kansas, in early March, before regrowth. Rhizomes on the surrounding soil (30 × 30 × 15 cm) were removed from the field and gently washed to free individual rhizomes from soil. Roots of each rhizome were trimmed to about 5 cm, and rhizome wet weights were measured. Sixty rhizomes of similar size and wet weight (about 0.89–1.21 g) were selected, each of which had at least 1 apparent shoot initial. The rhizomes were then transplanted, 1 per pot, into 25-cm diameter pots containing 3.5 kg (dry weight) Chase silty clay loam containing 7 $\mu g\ g^{-1}$ plant-available phosphorus (Bray test 1), freshly collected from the Konza Prairie Research Natural Area, Manhattan, Kansas. While one-third (20 pots) remained nonsterile, two-thirds of the pots (40) were filled with soil steamed at 80° C for 2 hours and allowed to cool for 48 hours. Thereafter, one-half of the pots containing steamed soil were inoculated with 400 freshly collected spores (per pot) of *Glomus etunicatum* Becker and Gerd., contained in 1 ml of water, pipeted onto the roots of rhizome at transplant. Spores were harvested from 'Piper' sudangrass (*Sorghum vulgare* var. *sudanense* (Hitch.) pot cultures and recovered by wet-sieving, decanting, and centrifuging in a 20-40-60% sucrose density gradient (Daniels and Skipper 1982). The remaining pots containing steamed soil and all pots containing nonsterile soil were not inoculated. In the present and the following experiments, *G. etunicatum* was used as mycorrhizal inoculum because it is a naturally occurring tallgrass prairie species and is highly efficient on big bluestem (Hetrick, unpublished).

Additional treatments for each soil (inoculated or noninoculated steamed soil, or nonsterile soil) with 5 replicate pots per treatment were: (1) fertilization with 40 ml/pot KH_2PO_4 solution (30 $\mu g\ g^{-1}$ P) pipeted onto the soil surface at transplant; (2) no fertilization; (3) P fertilization and application of 45 $\mu g\ g^{-1}$ benomyl fungicide (Benlate, 50% a.i.), applied as a soil drench 1 week following experimental set-up and reapplied every fourth week thereafter; and (4) no fertilization but benomyl was applied.

Since the larger roots were retained on the rhizomes used in this study to ensure their survival, even plants which were not inoculated with *G. etunicatum* were often mycorrhizal. Therefore, in a subsequent smaller study all roots were removed from each rhizome to eliminate the pre-existing mycorrhizal colonization.

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Thirty rhizomes of similar size and wet weight were selected, each containing at least 1 shoot initial. Twenty rhizomes were transplanted, 1 per pot, into 450 g (dry weight) steamed soil, while 10 rhizomes were transplanted into similar amounts of nonsterile soil contained in 6- × 25-cm pots. One-half of the seedlings planted in the steamed soil were inoculated with 400 spores/pot *G. etunicatum*, as previously described. There were 10 replicate pots within each of these treatments.

Both experiments were arranged in a completely randomized design in a greenhouse maintained at 15 to 25° C and fertilized biweekly with Peter's No-Phos (25-0-25) special fertilizer solution (Robert B. Peters Co., Inc., Allentown, Pa. 18014). Greenhouse lighting was supplemented with sodium vapor lights to maintain a 16-hour day length.

Plants in each experiment were harvested after 15 weeks and the roots washed free of soil. Shoots and roots were dried at 70° C for 3 days, after which shoot, root, and total dry weights were determined. The dried roots were subsampled, stained in trypan blue (Phillips and Hayman 1970), and examined microscopically to assess percentage root colonization using a petri plate scored into a 1-mm squares (Daniels et al. 1981). Differences in shoot, root, and total dry weights and percentage root colonization were subjected to analysis of variance ($P \leq 0.05$). Since significant interactions were again evident, main effects analyses were precluded and means were separated using least significant difference (LSD). Since shoot and root dry weights did not reveal treatment effects which were not also demonstrated by total plant dry weights, only total dry weights are presented. Correlation analysis was used to determine the relationship between initial rhizome wet wets and final shoot, root, and total plant dry weights and root colonization. Since initial wet weights were not correlated with the other parameters, the wet weight data were not presented.

To assess effects of repeated clipping (simulated grazing) and mycorrhizae on plant dry weight and mycorrhizal root colonization, big bluestem seedlings were planted in 6 × 25-cm pots containing 450 g (dry weight) steamed or nonsterile prairie soil. Sixty pots containing steamed soil were inoculated with 400 spores/pot *G. etunicatum* as described earlier, while 30 pots containing steamed soil and 60 pots containing nonsterile soil were not inoculated. The inoculated seedlings and the seedlings in nonsterile soil were subdivided into 2 groups of 30 pots. One subgroup was amended with 45 $\mu g\ g^{-1}$ benomyl, applied as a soil drench 6 weeks after experimental set-up. Because in preliminary experiments mycorrhizal root colonization was controlled equally well by four-week or six-week applications of benomyl, in this experiment benomyl was reapplied every sixth week, because this coincided with clipping and harvest dates. The other subgroup and all 30 noninoculated plants in steamed soil did not receive benomyl. Six pots of each treatment were harvested after 6, 12, 18, 24, and 30 weeks. At each harvest date, plant dry weight and root colonization of harvested plants were assessed as in the previous experiment, while the remaining plants were clipped to soil level and the clippings dried and weighed. Greenhouse conditions and fertilization schedule for this experiment were similar to those described previously. Data were subjected to analysis of variance, mean separation using LSD, and then displayed graphically using cubic spline curves (Motulsky 1987).

Results and Discussion

Influence of Mycorrhizae on Mature Plants

When the effect of mycorrhizae on big bluestem rhizomes was assessed, inoculation significantly ($P \leq 0.05$) increased biomass production in steamed soil whether or not plants were fertilized with P (Table 1). When inoculated plants were treated with benomyl fungicide, however, biomass of inoculated and noninoculated plants grown in steamed soil was similar. In nonsterile soil, biomass production of fertilized and unfertilized plants and fungicide-treated fertilized plants was similar. Without P fertilizer, however, fungicide-treated plants were smaller, and were similar to noninoculated plants grown in steamed soil.

Table 1. Influence of mycorrhizae and P fertilization on biomass production and mycorrhizal root colonization of big bluestem plants regrown from rhizomes.

Soil treatment	Total dry wt(g)[1]	Root colonization(%)[1]
Steamed soil-		
No fungicide		
Inoculated		
No P	18.30^{ab}	60.61^{a}
P[2]	21.14^{a}	31.40^{b}
Noninoculated		
No P	9.30^{cde}	4.20^{e}
P	11.88^{bcde}	5.40^{de}
Fungicide treated[3]		
Inoculated		
No P	6.58^{ed}	18.6^{bcd}
P	17.74^{ab}	21.8^{bc}
Noninoculated		
No P	5.51^{e}	5.6^{de}
P	12.49^{bcd}	5.4^{de}
Nonsterile soil-		
No fungicide		
No P	14.38^{abc}	20.8^{bc}
P	16.73^{ab}	19.0^{bcd}
Fungicide treated[3]		
No P	6.75^{de}	8.2^{de}
P	16.57^{ab}	11.0^{cde}

[1]Means followed by the same letters are not significantly ($P>0.05$) different as determined using LSD. Since plants were regrown from mature rhizomes, roots attached to the rhizomes were already colonized by indigenous mycorrhizal fungi.
[2]Fertilized with 30 $\mu g\ g^{-1}$ as KH_2PO_4.
[3]Treated with 45 $\mu g\ g^{-1}$ benomyl.

Similar trends were reflected in root colonization. Noninoculated plants maintained low levels of root colonization even in steamed soil, presumably because rhizomes were already colonized before they were removed from the field for use in the present experiment. Greater root colonization occurred in response to inoculation in steamed soil as compared with noninoculated or fungicide-treated plants. Fungicide treatment reduced root colonization of inoculated plants in steamed soil and in nonsterile soil, but did not entirely inhibit root colonization in any treatment. Low levels of root colonization are often evident after fungicide treatment even though the symbiosis has been inactivated by the fungicide (Kough et al. 1987). Phosphorus fertilization reduced root colonization only for inoculated plants grown in steamed soil.

When plant roots were removed from big bluestem rhizomes before planting, at harvest time inoculated plants averaged 2.17 g biomass while noninoculated plants were significantly (LSD = 0.36) smaller and averaged 0.14 g/plant. Plants grown in nonsterile soil, however, produced an intermediate, different amount of biomass, 0.91 g. Root colonization was high in inoculated plants (77.71%), significantly (LSD = 4.46) lower in plants grown in nonsterile soil (22.29%), and noninoculated plants in steamed soil were not colonized. The lack of colonization in noninoculated plants in steamed soil following removal of roots suggests that mycorrhizal fungi do not colonize rhizomes. These colonization levels are similar to those obtained in the previous experiment when roots were not removed from rhizomes. In contrast, however, biomass produced from rhizomes with intact roots far exceeded that from rhizomes with roots removed in the present experiment. Presumably, reestablishment of the root system was

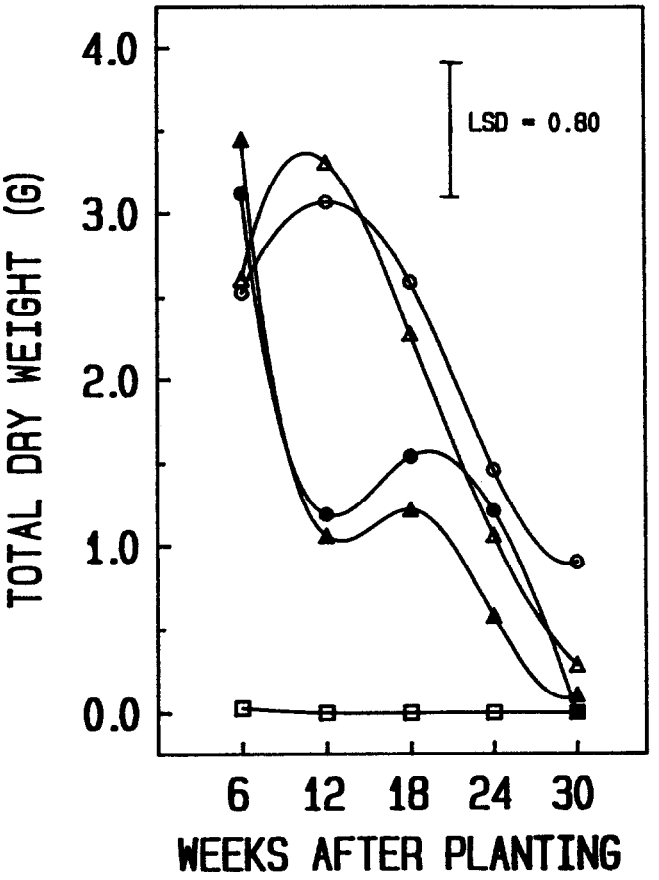

Fig. 1. Mean dry weight of repeatedly clipped big bluestem plants grown in steamed soil without mycorrhizal fungus inoculation (□), with incoulation but without benomyl fungicide (Δ), with inoculation and benomyl application (▲), in nonsterile soil without inoculation or benomyl fungicide (○), and in nonsterile soil without inoculation but with benomyl treatment (●). Plants were harvested and benomyl 45 $\mu g\ g^{-1}$) was applied at 6 weeks and every 6 weeks thereafter.

metabolically costly and plant biomass development was slowed or restricted. Despite the smaller size of plants regrown from rhizomes from which roots were removed, biomass production was higher in mycorrhizal plants than in nonmycorrhizal plants.

Influence of Mycorrhizae on Grazing Tolerance

At the first harvest, 6 weeks after the experiment was initiated, dry weight of inoculated plants and those grown in nonsterile soil were similar (Fig. 1). Noninoculated plants in steamed soil did not grow, demonstrating the exceptionally high dependence of this species on mycorrhizal symbiosis. In response to the first fungicide treatment (applied at 6 weeks), fungicide-treated plants harvested after 12 weeks were smaller then inoculated plants or those grown in nonsterile soil without fungicide treatment. With successive fungicide treatments and harvests, differences between fungicide-treated mycorrhizal plants and untreated mycorrhizal plants diminished. Unlike the plants regrown from rhizomes in the previous experiment, in the present experiment, where plants were grown from seedlings, noninoculated plants grown in steamed soil could not survive without mycorrhizae. Apparently, mycorrhizae increase biomass production, but this positive effect can be lost when plants are exposed to intensive stress from successive clipping treatments.

When biomass production of shoots and roots were considered separately, shoots of mycorrhizal plants (inoculated plants in steamed soil and plants grown in nonsterile soil) regrew after the first clipping at 6 weeks and produced similar biomass by 12 weeks

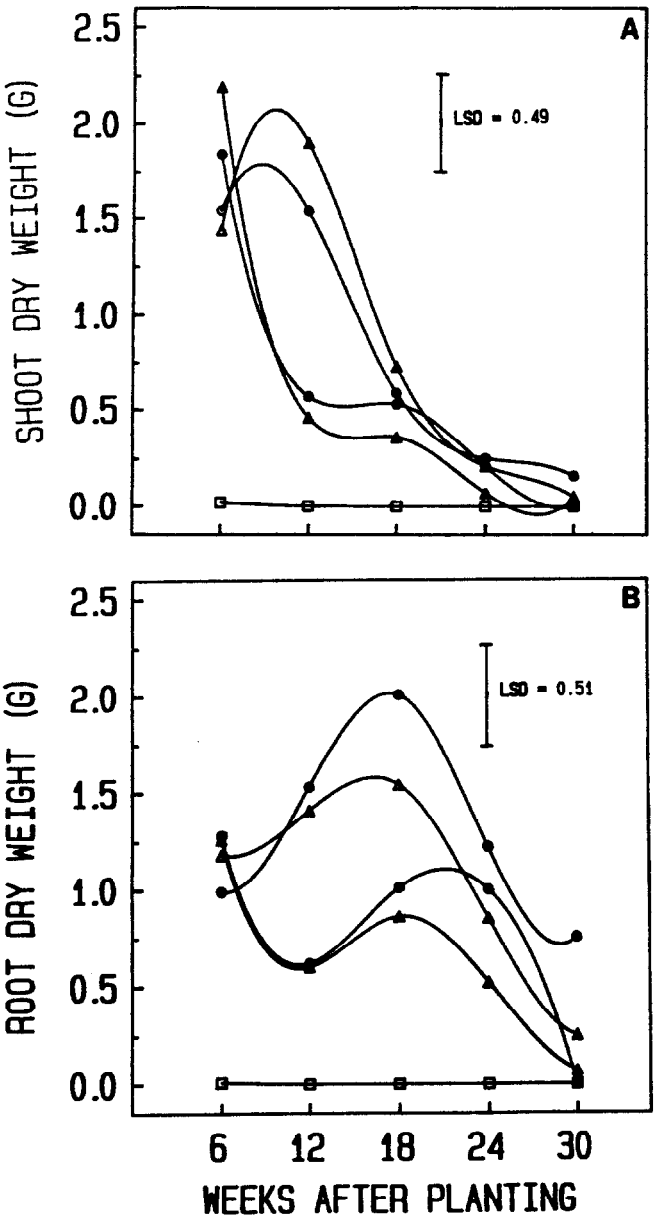

Fig. 2. Mean shoot dry weight (A) and mean root dry weight (B) of repeatedly clipped big bluestem plants grown in steamed soil without mycorrhizal fungus inoculation (□), with inoculation but without benomyl fungicide (Δ), with inoculation and benomyl application (▲), in nonsterile soil without inoculation or benomyl fungicide (○), and in nonsterile soil without inoculation but with benomyl treatment (●). Plants were harvested and benomyl 45 $\mu g\ g^{-1}$) was applied at 6 weeks and every 6 weeks thereafter.

(Fig. 2a). In contrast, shoot biomass of nonmycorrhizal (fungicide-treated) plants declined after the first harvest and did not regrow at any harvest thereafter. Shoot biomass of plants in all treatments was similar at the 18, 24, and 30-week harvests. Thus, maximum shoot production occurred for mycorrhizal plants at the first and second harvests, with mycorrhizal growth response absent thereafter. Root biomass of mycorrhizal plants increased after the first and second harvest, with maximum root biomass apparent at the third harvest (Fig. 2b). In contrast, root biomass produced by fungicide-treated plants declined between the first and second harvest, and was negligible by the fifth harvest.

Therefore, since noninoculated plants in steamed soil did not survive, they were excluded from consideration of root/shoot ratios. When the effect of mycorrhizae on root/shoot ratios of the

remaining 4 soil/mycorrhizae treatments were compared, there was no significant ($P \leq 0.05$) effect of soil treatments on root/shoot ratio. Therefore, data for the soil mycorrhizae treatments were combined for main effects comparison of change in root/shoot ratio at the various harvests. Root/shoot ratios of the combined soil/mycorrhizae treatments were 0.68, 1.02, 2.45, and 5.32, and 2.18 at the 6, 12, 18, 24, and 30-week harvests, respectively. Thus, root/shoot ratio increased with increasing clipping pressure, with the root/shoot ratio at the 24-week harvest significantly greater than the others (LSD = 1.79). Apparently, these changes in root/-shoot ratio are not caused by mycorrhizae but occur in response to clipping stress. The changes in root/shoot ratio probably reflect reallocation of resources to sustain root production at the expense of shoot production in response to clipping stress. The main effect of mycorrhizae, then, appears to be increased root and shoot biomass, but this growth response diminishes as stress increases.

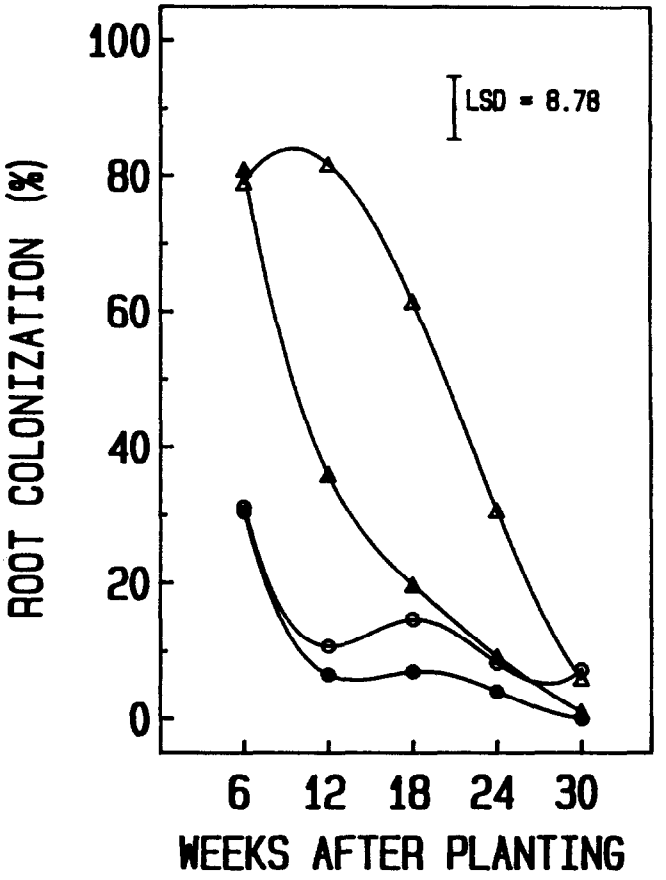

Fig. 3. Mycorrhizal root colonization of repeatedly clipped big bluestem plants grown in steamed soil, with inoculation, without benomyl fungicide (Δ), with inoculation and benomyl application (▲), in nonsterile soil without inoculation or benomyl fungicide (○), and in nonsterile soil without inoculation but with benomyl treatment (●). Plants were harvested and benomyl (45 $\mu g\ g^{-1}$) was applied at 6 weeks and every 6 weeks thereafter. Noninoculated plants grown in steamed soil were not colonized and were, therefore, excluded from the figure.

By the fourth and fifth harvests clipping pressure was apparently so severe that mycorrhizal benefit to shoot biomass production was eliminated, perhaps because there was no more P available to mycorrhizal than to nonmycorrhizal plants. It seems more likely, however, that under severe clipping pressure, plant photosynthesis was no longer sufficient to support the fungi. This latter hypothesis was supported when root colonization was examined.

At the first harvest, mycorrhizal colonization of roots was higher in steamed than in nonsterile soil (Fig. 3). While fungicide application significantly reduced root colonization in steamed soil, root colonization in fungicide-treated plants in steamed soil still exceeded root colonization of plants grown in nonsterile soil with or without fungicide at 12 weeks. Fungicide treatment of plants in steamed soil did not entirely eliminate root colonization, although root colonization decreased steadily in response to successive applications of the fungicide or in response to each clipping. Since growth response was inhibited by fungicide application to plants in steamed soil (Fig. 1), it seems likely that symbiotic function was impaired by the fungicide even if colonization structures had not fully disappeared from the root system in response to fungicide treatment. Root colonization of fungicide-treated and untreated plants in nonsterile soil was similar. The lower colonization levels of plants in nonsterile soil has been frequently observed and attributed to competition between mycorrhizae and soil microorganisms for nutrients (reviewed in Hetrick 1989).

While colonization levels in all other treatments generally declined after clipping, colonization of plants in steamed soil was maintained after the first clipping and declined only after the second clipping. By the fifth harvest, <8% colonization existed in all treatments.

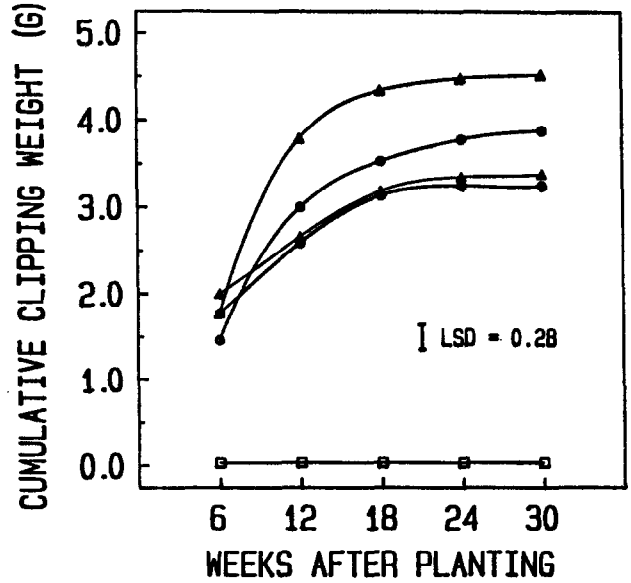

Fig. 4. Cumulative shoot biomass of repeatedly clipped big bluestem plants grown in steamed soil without mycorrhizal fungus inoculation (□), with inoculation but without benomyl fungicide (Δ), with inoculation and benomyl application (▲), in nonsterile soil without inoculation or benomyl fungicide (○), and in nonsterile soil without inoculation but with benomyl treatment (●). Plants were harvested and benomyl (45 $\mu g\ g^{-1}$) was applied at 6 weeks and every 6 weeks thereafter.

The influence of mycorrhizae on cumulative shoot biomass produced was assessed using the plants harvested at 30 weeks (Fig. 4). By the end of the experiment, inoculated plants grown in steamed soil produced more shoot biomass than plants grown in nonsterile soil and these mycorrhizal plants (those grown in inoculated steamed soil or in nonsterile soil) produced more biomass than fungicide-treated plants in either of these soils. Plants grown from the beginning of the experiment without mycorrhizae (noninoculated plants grown in steamed soil) did not grow and produced virtually no shoot biomass. For these plants, mycorrhizae are essential to survival. Plants in all other treatments were exposed, at least initially, to mycorrhizae since the first fungicide treatment occurred after the 6-week harvest. This explains why all plants initially exposed to mycorrhizae were of similar size at the first harvest. Presumably, the root development which occurred at 6 weeks allowed further plant growth, even in treatments where

fungicide application limited mycorrhizal nutrient uptake. The greater shoot biomass production of mycorrhizal plants, however, demonstrates the stimulatory effect of the symbiosis even on plants which have already initiated root development.

Mycorrhizal fungi may be particularly beneficial to plants when plants are stressed, e.g., during seedling establishment or flowering (McGonigle and Fitter 1988), under drought stress (Stahl and Smith 1984) or under clipping stress (Wallace 1981, Wallace and Svejcar 1987). Our studies demonstrate that regrowth of big bluestem in spring from mature rhizomes also depends upon mycorrhizal symbiosis, even though high quantities of nutrients are stored within the rhizomes. Apparently, nutrients stored in rhizomes are not sufficient to support plant growth without mycorrhizae, but they probably are sufficient to initiate regrowth and allow time for the symbiosis to reestablish and begin functioning. This would explain the greater growth of plants from rhizomes with intact root vs. rhizomes from which we removed all roots, and the greater growth of inoculated rhizomes or those not treated with fungicide than those without mycorrhizae. Therefore, the importance of mycorrhizal to tallgrass prairie plants is certainly not limited to seedling establishment, although the importance of the symbiosis in seedling establishment is considerable (Hetrick et al. 1989).

Other studies of mycorrhizal benefit to tallgrass prairie plants (reviewed in Hetrick 1989) demonstrated that mycorrhizal symbiosis is suppressed in nonsterile soil. The suppression may result from direct competition between soil microorganisms and mycorrhizae. The reduced growth and lower root colonization of big bluestem from rhizomes in nonsterile soil demonstrates that nonsterile soil suppresses growth of mature plants as well as seedlings and underscores the importance of the symbiosis to rhizome regrowth.

In previous studies of mycorrhizal effects on grazing tolerance, plant growth in fumigated soil (mycorrhizae absent) was compared with growth in nonsterile soil (mycorrhizae present), making it difficult to distinguish mycorrhizal effects from those of other soil microorganisms (Wallace 1981, Wallace 1987, Wallace and Svejcar 1987). The present studies confirm that mycorrhizae can improve herbage yield under clipping pressure, but this beneficial effect of mycorrhizae is lost when clipping pressure is severe. Under severe clipping intensity, mycorrhizal root colonization and growth response were lost. Under severe stress, plant resources of both mycorrhizal and nonmycorrhizal plants are reallocated, i.e., root growth is sustained while shoot development is retarded. Similar negative effects of intensive grazing on mycorrhizal symbiosis and root to shoot ratio were reported by Bethlenfalvay and Dakessian (1984) and Bethlenfalvay et al. (1985). It is unclear, however, whether mycorrhizal and nonmycorrhizal plants undergo similar changes in root/shoot ratio because clipping stress precludes mycorrhizal function, forcing mycorrhizal and nonmycorrhizal plants to employ similar survival strategies.

Intensive clipping of tallgrass prairie for even 1 year decreases tillering, herbage yield, rhizome nitrogen, and total nonstructural carbohydrate percentages in the subsequent season (Owensby et al. 1974). Our studies and those of Bethlenfalvay and Dakessian (1984) and Bethlenfalvay et al. (1985) suggest that overgrazing is also detrimental to mycorrhizal symbiosis. Presumably, a reduction in photosynthetic surface negatively affects the symbiosis. In this way, the deleterious effects of overgrazing in plants are compounded by the loss of the symbiosis. Since mycorrhizae are critical to mature plants, particularly to regrowth from rhizomes in spring, their loss from plants due to overgrazing may seriously reduce plant fitness and survival in subsequent season(s).

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