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EVIDENCE OF A MYCORRHIZAL MECHANISM FOR THE ADAPTATION OF *ANDROPOGON GERARDII* (POACEAE) TO HIGH- AND LOW-NUTRIENT PRAIRIES[1]

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Andropogon gerardii seed obtained from Kansas and Illinois was grown in a controlled environment in their own and each other's soils, with and without arbuscular mycorrhizal fungi (AMF). Each ecotype grew comparatively better in its own soil indicating adaptation to its soil of origin. Overall, *A. gerardii* benefited more from AMF in low-nutrient Kansas soil than Illinois soil. The two ecotypes, however, did not benefit equally from mycorrhizal infection. The Kansas ecotype was three times more responsive to mycorrhizal infection in the Kansas soil than was the Illinois ecotype. Our results indicate that plant adaptation to the nutrient levels of their local soils is likely to be due, at least in part, to a shift in their dependence on mycorrhizal fungi. The Illinois ecotype of *A. gerardii* has evolved a reduced dependence upon these fungi and greater reliance on a more highly branched root system. In contrast, the Kansas ecotype had a significantly coarser root system and invested proportionately greater carbon in the symbiotic association with AMF as measured by spore production. This study provides the first demonstration that plants can adapt to changing soil nutrient levels by shifting their dependence on AMF. This result has broad implications for our understanding of the role of these fungi in agricultural systems.

Key words: adaptation; *Andropogon gerardii*; mycorrhizae; Poaceae, prairie; soil phosphorus.

The adaptation of plants to aspects of their local environment is well documented (e.g., Clausen, Keck, and Hiesey, 1947). Of the many components of the plant's environment, the best examples of plant adaptation are to soil conditions, including the concentrations of specific ions (e.g., Antonovics and Bradshaw, 1970; Antonovics, Bradshaw, and Turner, 1971; Bradshaw, 1984) and overall soil fertility (Snaydon and Bradshaw, 1962; McGraw and Chapin, 1989). For many plant species uptake of soil minerals is facilitated by association with symbiotic fungi. Most plant species associate with arbuscular mycorrhizal fungi (AMF), which facilitate plant uptake of nutrients, especially phosphorus (Smith and Read, 1997). Therefore, the adaptation of plants to soils of varying fertility could be through an alteration of their relationship with these mycorrhizal fungi.

While plants differ in their dependence upon AMF independent of soil nutrient levels, generally, plant reliance on mycorrhizal fungi varies with soil nutrient concentration. In low-nutrient soils many plants are unable to grow without this association (e.g., McGee, 1985; Grime et al., 1987), but in high-nutrient soils many plants are less dependent on mycorrhizal fungi for nutrient uptake. A soil's phosphorus supply rate is often the primary determinant of plant dependence on mycorrhizal fungi (Gerdemann, 1975). In fact, the benefit of AMF to plants changes with the amount of available soil phosphorus (e.g., Hetrick, Wilson, and Cox, 1992; Hetrick, Wilson, and Todd, 1996). When soil phosphorus is abundant, the cost of the mycorrhizal association can outweigh the benefit, and plant growth may be reduced by the association with the fungus (e.g., Bethlenfalvay, Brown, and Pacovsky, 1982; Hetrick, Wilson, and Todd, 1992; Hetrick, Wilson, and Cox, 1993). From this observation, one might expect that in high-phosphorus soils, plants that are less dependent on AMF will lose proportionally less carbon to the fungus and, therefore, have greater fitness than plants with a strong dependence on these symbiotic fungi.

As a result of variation in dependence among plant species and plant genotypes on AMF (e.g., Bryla and Koide, 1990; Hetrick, Wilson, and Cox, 1992, 1993; Hetrick, Wilson, and Todd, 1992; Wilson and Hartnett, 1998), one might expect to find fewer mycorrhiza-dependent plant species under high-nutrient conditions. Indeed this pattern has been observed for shifts in species composition, where less mycorrhizal dependent plant species have been observed to increase as a result of phosphorus fertilization (e.g., Medve, 1984). However, an analogous shift in the genetic composition of plant populations to genotypes with lower dependence on mycorrhizal fungi in high fertility soils has not been examined.

In this study, we tested the hypothesis that plant populations adapt to differences in soil fertility by altering their dependence on mycorrhizal fungi. We took advantage of a well-studied plant species, *Andropogon gerardii* Vitman, which is the dominant grass species of the North American tallgrass prairie and is known to vary in its dependence on mycorrhizal fungi. Specifically, *A. gerardii* has been found to be highly dependent on mycorrhizal fungi in the low-phosphorus soils of Kansas (e.g., Hetrick, Wilson, and Todd, 1992; Wilson and Hartnett, 1998) but is not dependent on mycorrhizal fungi in the high-phosphorus soils of Illinois (Bentivenga, 1988; An-

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TABLE 1. Soil texture, available P, and extractable N contents of the Illinois and Kansas, USA, soils at harvest.

Soil site	Soil texture	Bray-1 phosphorus (μg/g)	NH_4-N (μg/g)	NO_3-N (μg/g)	Mineral N (μg/g)
Illinois	loam	22.1	5.7	0.6	6.3
Kansas	clay loam	10.2	1.4	3.9	5.3

derson, Hetrick, and Wilson, 1994). We tested whether this difference in dependence on mycorrhizal fungi results from ecotypic differentiation of *A. gerardii*.

MATERIALS AND METHODS

Study system—We evaluated the responses of two ecotypes of *A. gerardii* to inoculation with mycorrhizal fungi in high- and low-phosphorus soils in a full factorial experiment with two levels of plant ecotype (Illinois and Kansas), mycorrhizal fungi (inoculated and uninoculated), and soil fertility (Illinois and Kansas). Specifically, we used *A. gerardii* and soil from the Flint Hills of Kansas and from the restored prairie at the Fermilab National Environmental Research Park in Batavia, Illinois (Jastrow, 1987). The Illinois soil had >2.5 times as much available phosphorus as the Kansas soil (Table 1). Levels of mineral nitrogen (NO_3 and NH_4) also varied but not as widely (Table 1). This system was used to test whether two populations of *A. gerardii* have adapted to their respective soil environments and whether a shift in mycorrhizal dependence contributes to this adaptation.

Seed—Seed of *A. gerardii* var. Kaw was obtained by the Soil Conservation Service from a composite of lines selected after four or more generations from progeny of 200 accessions collected in 1935 in native Flint Hills grasslands (Kansas Agricultural Experimental Station, Agronomy Department, Manhattan, Kansas, USA). *Andropogon gerardii* seed from Illinois was hand collected at Fermilab National Environmental Research Park in Batavia, Illinois, USA.

Soil preparation—Soil (surface 20 cm) was taken from Fermilab and from the Konza Prairie Research Natural Area, air dried, and passed through a 6.25-mm mesh sieve. The soils were uniformly moistened and pasteurized by steaming for 1–1.5 hr, cooled, remoistened, mixed, and steamed again. After air drying, the Illinois soils were mixed with calcined clay (Terragreen Soil Conditioner, Oil-Dri Corporation of America, Chicago, Illinois, USA) and flint sand (0.45–1.20 mm effective diameter size) in a ratio of 3 : 1 : 1 by volume. For the Kansas soils, coarse sand (1.20–1.50 mm), which had the same effective diameter size as calcined clay, replaced the clay in the potting mix. Soil texture, therefore, between Illinois and Kansas soil was similar. Sand was substituted for calcined clay in the Kansas soils to limit any addition of P in Kansas soil. Illinois and Kansas soils were amended to increase the porosity of soils that inevitably become compacted in pot experiments.

Inoculum—We used inoculum established by Gail Wilson from soil obtained within the Konza Prairie Research Natural Area. We found that this culture was a mixture of *Glomus mosseae*, *G. occultum*, *G. microaggregatum*, and *G. geosporum*. The inoculum was amplified by adding a small amount to pots filled with a 1 : 1 mix of pasteurized Illinois soil and sand and planted with *A. gerardii* and *Sorghum vulgare*. After the host plants had grown for 4 mo, the aboveground portion of the plants were removed, and the soil, including roots, was homogenized by cutting roots into small fragments and mixing thoroughly with soil. The inoculum was stored at 4°C for 3–6 mo prior to use.

Seedling establishment and plant growth—Before planting, 6 × 15 cm containers were filled with 500 cm^3 of pasteurized Kansas or Illinois soil mix. For mycorrhizal treatments, 25 cm^3 of inoculum was layered on top of the pasteurized soil mix and covered with another 25 cm^3 of pasteurized soil mix. For nonmycorrhizal treatments, 50 cm^3 of the appropriate (Kansas or Illinios) pasteurized soil mix was added to the initial 500 cm^3. Treatments were replicated six times with Kansas and Illinois isolates in their own soils and five times in treatments with Illinois isolates in Kansas soil and Kansas isolates in Illinois soil. Soil flora minus the mycorrhizal component was added back by mixing unpasteurized soil from either Kansas or Illinois with deionized water, sieving the suspension through a 38-μm mesh sieve, and adding 50 mL of the wash to pots containing Kansas or Illinois soil, respectively. Two-week-old seedlings of *A. gerardii* from either Kansas or Illinois were transplanted into appropriate pots and grown in an environmental chamber on a 16 : 8 h day : night cycle. Day and night temperatures were set at 28° and 22°C, respectively. Daily photosynthetically active radiation (PAR) averaged 433 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at harvest. Relative humidity was maintained at ~50%. The plants were watered with deionized water to field capacity three times per week and grown for 74–76 d.

Harvest—Before harvesting the plants, two soil cores (1.4 × 8 cm) were removed from each pot and frozen for later determination of external mycorrhizal hyphal lengths. Plants and soil were then removed from the pots. After carefully separating the roots from the soil mix, the roots were gently washed under a stream of running water over a 1-mm mesh sieve. Roots were cut away from shoots, separated into coarse (>1.0 mm diameter) and fine (≤1.0 mm diameter) categories, and blotted to remove surface water. Fine roots were cut into 2-cm segments and mixed thoroughly. A 0.25-g subsample was removed for determination of fine root length and mycorrhizal colonization, and the fresh mass of the remaining fine roots was determined. Coarse roots, the remaining fine roots, and shoots were then dried to constant mass at 65°C, and weighed. Total root dry mass was calculated by using the dry : fresh mass ratio of the remaining fine roots to estimate the dry mass of the subsample removed to assay root length and colonization.

Soils analyses—After harvest soil samples from each soil treatment were analyzed for available phosphorus and mineral nitrogen at the Kansas State University Soil Testing Laboratory. Soil phosphorus was determined by the Bray P1 method. For nitrate-nitrogen and ammonia-nitrogen, soil samples were extracted for 30 min with 1 mol/L KCl and analyzed colorimetrically. Samples from each treament were pooled. The average of the P, NH_4, NO_3, and mineralized N in Illinois soil treatments and Kansas soil treatments are presented in Table 1.

Estimation of AMF, root length, and root architecture—The 0.25-g subsample of fine roots was cleared with potassium hydroxide and stained with trypan blue (Reinhardt and Miller, 1990), and internal AMF infection was determined (McGonigle et al., 1990). The length of external hyphae was determined by membrane filtration (Miller, Reinhardt, and Jastrow, 1995) for the soil subsamples removed just prior to plant harvest. Two replicates for each pot were assayed and averaged, and the length of hyphae produced in each pot was calculated. The number of spores per pot was estimated from a 50-mL subsample of homogenized soil by using sucrose-density gradient centrifugation (Daniels and Skipper, 1982). Spore volume was determined by inserting the average radius of the spores for each species into the formula for a sphere ($4/3\pi r^2$). The average radii of *G. occultum* and *G. microaggregatum* were 35 and 15 μm, respectively.

An Ag-vision (Decagon Devices, Pullman, Washington, USA) image analysis system was used to estimate the lengths of the stained 0.25-g subsamples of fine roots by the digital line intercept method (Harris and Campbell, 1989). Root lengths per pot were calculated by using the dry : fresh mass ratio and the mass of the remaining roots. The number of branches in the stained subsamples was counted under a dissecting microscope. The ratio of root branches to root length was calculated for each subsample from the mycorrhizal treatments. Branching ratios were not calculated from the nonmycorrhizal treatments because of the scarcity of roots in the nonmycorrhizal treatment in Kansas soil. Mycorrhizal response was calculated as follows: (dry mass inoculated − dry mass uninoculated)/dry mass uninoculated.

Statistical analysis—The natural log of the total plant dry mass was analyzed with a three-way analysis of variance by using the general linear models

TABLE 2. The results of the analysis of variance of the effects of plant ecotype, soil type, and presence or absence of arbuscular mycorrhizal fungi (AMF) on *Andropogon gerardii* biomass and root : shoot ratio.

Effect	df	Plant biomass SS	Root : shoot SS
Ecotype	1	0.0713*	0.0040NS
Soil	1	14.9304****	0.0945NS
AMF	1	1.6899****	0.3443**
Ecotype × AMF	1	0.1982****	0.0164NS
Soil × AMF	1	2.2014****	0.2908**
Soil × Ecotype	1	1.6509****	0.1529*
Soil × Ecotype × AMF	1	0.1782***	0.0056NS
Error	28	0.2888	0.7483

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS = not significant.

procedure of SAS (SAS, 1986). We transformed the data to improve the homogeneity of variance. We were particularly concerned with tests of ecotypic differences in growth. Local adaptation for the soil was tested with the plant ecotype × soil source interaction. Differences in overall mycorrhizal dependence between the plant ecotypes were tested with the plant ecotype × mycorrhizae interaction. We specifically predicted that the Kansas populations would be more responsive to mycorrhizal inoculum than the Illinois population. Finally, the differences in environmental shifts of these two ecotypes were tested by the three-way interaction between plant ecotype × mycorrhizae × soil.

We also used factorial analyses of variance as above to investigate shifts in the allocation patterns of the plants. We specifically tested for changes in root : shoot ratios, colonized root length, density of external hyphae, and volume of spores produced. Root : shoot ratios, external hyphal length colonized root lengths, and spore biovolume were natural log transformed prior to analysis.

We tested for potential shifts in the composition of AMF during the course of the experiment using multivariate profile analysis on ranks of spore counts of individual species (as developed by Bever et al., 1996). In this analysis, the significance of the profile interaction in each term in the model statement tests for changes in community composition corresponding to that term. For example, the significance of the profile interaction with plant population tests for changes in fungal community composition between the two ecotypes during the course of the experiment. The multivariate analysis of variance was followed by univariate analyses of the ranks of spore counts of individual fungal species.

RESULTS

Biomass—The Illinois ecotype generally grew larger than the Kansas ecotype and both ecotypes grew larger in Illinois than Kansas soil. The significant interaction between plant ecotype and soil type confirms that the growth rate of *A. gerardii* depends upon the soil in which they are grown (Table 2). In fact, the biomass of the Kansas ecotype is significantly greater than the biomass of the Illinois ecotype in Kansas soil. The reverse is true in Illinois soil. These data demonstrate that each ecotype grew significantly better in their own soil, thus confirming local adaptation and ecotypic differentiation (Fig. 1).

Overall, mycorrhizal inoculation had a positive effect on plant growth. However, the level of growth promotion was dependent on the soil in which the plants were grown as indicated by the significance of the mycorrhizae × soil interaction (Table 2). Mycorrhizal inoculation improved growth in Kansas soil, but not in Illinois soil (Fig. 1).

The Kansas ecotype generally benefited more from inoculation with mycorrhizal fungi than the Illinois ecotype, as indicated by the significant plant ecotype × mycorrhizal fungi interaction (Table 2). Moreover, as the significant three-way interaction indicates (Table 2), these ecotypes differed in the environmental dependence of their response to mycorrhizal inoculum. Specifically, the *A. gerardii* ecotype from Kansas benefited more from mycorrhizal inoculation than the Illinois ecotype in the Kansas soil. In Illinois soil, however, neither ecotype benefited from mycorrhizal infection (Fig. 1).

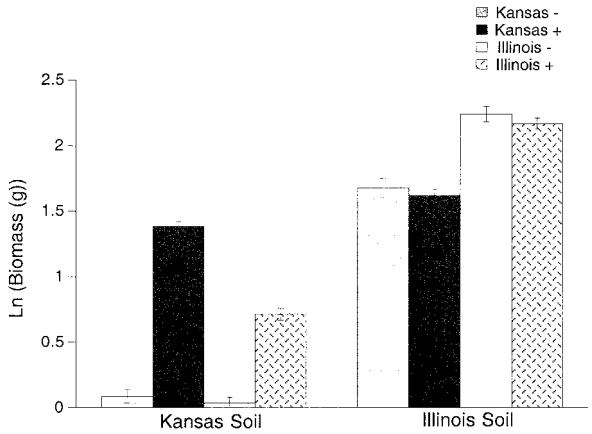

Fig. 1. The natural log average dry mass (in grams) of the Kansas and Illinois ecotypes of *Andropogon gerardii* grown in Kansas and Illinois soil with (+) and without (−) AM fungal inoculation are shown. Bars indicate ±1 SE.

Allocation below ground—The root : shoot ratios of *A. gerardii* were dependent upon the mycorrhizal status of the plants and the soil in which they were grown (Table 2). Mycorrhizal plants had a higher root : shoot ratio than nonmycorrhizal plants in Kansas soil, but there was no significant difference in Illinois soil (Fig. 2). The root : shoot ratios did not differ significantly between the two plant ecotypes overall, but root : shoot ratios were significantly affected by the interaction between plant ecotype and soil origin (Table 2). This effect was due to the lesser overall response of the Kansas ecotype to the differing nutrient conditions in the Kansas and Illinois soils (Fig. 2).

The branching pattern of the roots was strongly dependent upon the ecotype of *A. gerardii* (Table 3). The Illinois ecotype had significantly more branching than the Kansas ecotype (Fig. 3A) indicating a shift in root architecture from a coarse root

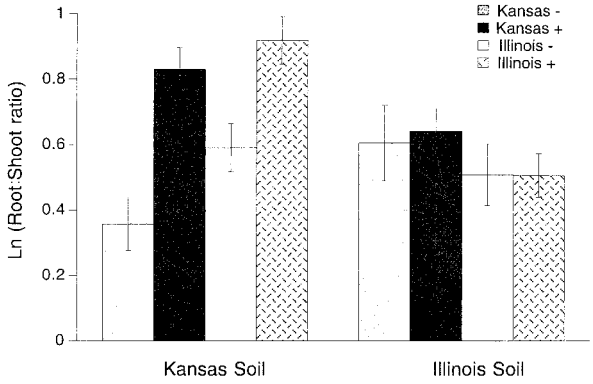

Fig. 2. The natural log of root : shoot ratio of the Kansas and Illinois ecotypes of *A. gerardii* grown in Kansas and Illinois soil. Bars indicate ±1 SE.

TABLE 3. The results of analysis of variance of root branching, infected root length, external mycorrhizal hypha, and total spore volume from each plant population. The log of root branching, infected length, external hyphae, and spore volume was analyzed.

Effect	df	Root branching SS	Infected root length SS	External hyphae SS	Spore volume SS
Soil	1	0.0028NS	3.49****	1.545**	27.22****
Ecotype	1	0.0365**	0.16NS	0.596NS	10.32****
Ecotype × Soil	1	0.0135NS	1.86**	0.228NS	0.91NS
Error	18	0.0744	2.57	2.793	3.92

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS = not significant.

TABLE 4. The results of analysis of variance of spore density of *Glomus occultum* and *G. microaggregatum* associated with each plant population. These analyses were performed on the rank of spore counts.

Effect	df	*G. occultum* SS	*G. microaggregatum* SS
Soil	1	38.78NS	609.02****
Ecotype	1	509.69****	149.39***
Ecotype × Soil	1	88.73*	6.60NS
Error	18	216.93	168.97

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS = not significant.

system in the Kansas ecotype to a finer root system in the Illinois ecotype.

Plant allocation to mycorrhizal fungi diverged between the two ecotypes of *A. gerardii*. There were no overall differences in the length of infected root between the two ecotypes, however there were large differences in the infected root length between the soil types and a significant interaction between soil type and plant ecotype (Table 3). The significance of this interaction term results from the Illinois ecotype having a relatively greater infected root length in Illinois soil, while the Kansas ecotype had a relatively greater infected root length in the Kansas soil (Fig. 3B). The density of external hyphae was not significantly different between the populations (Table 3). The density of external hyphae was, however, strongly influenced by the soil in which the plants were grown with the density being higher in the Illinois soil (Fig. 3C). The strongest effect of *A. gerardii* ecotype on mycorrhizal structures was on total spore biovolume ($F_{1,18} = 47.0$, $P < 0.0001$, Table 3). Much greater spore biovolume was produced when plants were grown in the Kansas soil than the Illinois soil and when plants were grown in association with the Kansas ecotype compared to when they were grown with the Illinois ecotype (Fig. 3D).

AM fungal community composition—While AM fungal composition was initially similar across treatments, the composition of the AM fungal community, as estimated by the density of freshly produced spores, changed during the course of the experiment (Table 4). Community composition was significantly affected by the soil and most dramatically changed by the plant ecotype. *Glomus occultum* sporulated more profusely with the Kansas ecotype, while *G. microaggregatum*

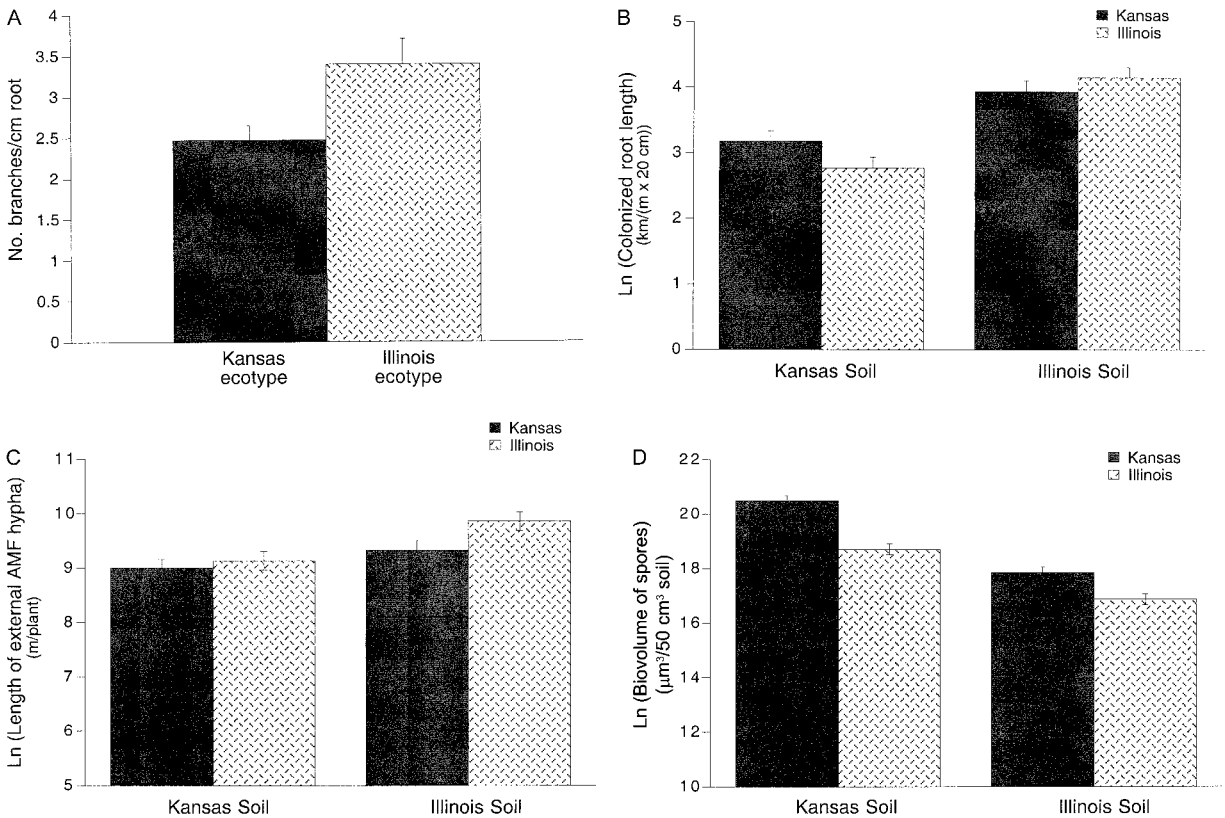

Fig. 3. Root-branching, percentage of infection, density of external hyphae, and spore biovolume are presented for the two ecotypes in both soils. Bars indicate ±1 SE. (A) Root branching per centimeter of root length. (B) The length of roots infected with mycorrhizal fungi. (C) Density of external hyphae. (D) Biovolume of AM fungal spores.

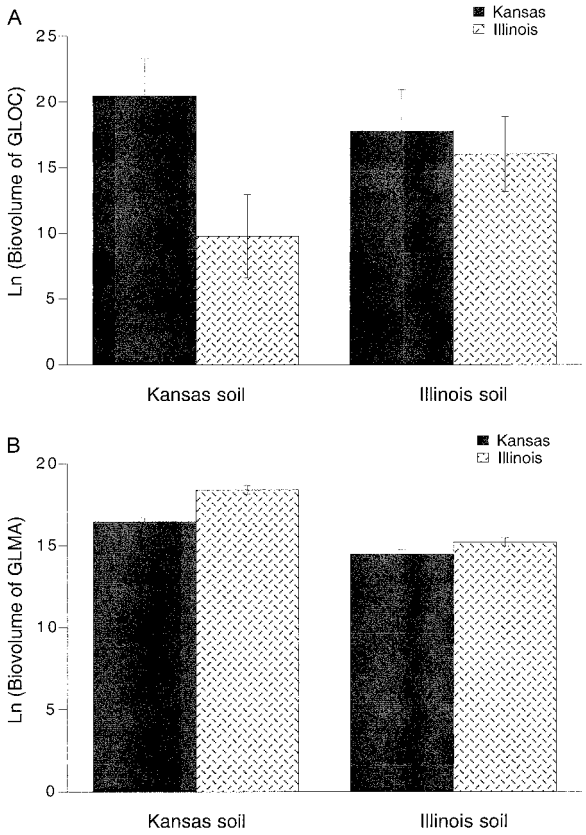

Fig. 4. Spore biovolume for two of the AM fungal species are presented (volume was determined by inserting the average radius of the spores for each species into the formula for a sphere [$4/3\pi r^2$]). Bars indicate ± 1 SE. (A) The log of the spore volume of *G. occultum* (GLOC) in Kansas and Illinois soils associated with the two ecotypes ($r = 35$ μm). (B) The log of the spore volume of *G. microaggregatum* (GLMA) in Kansas and Illinois soils associated with the two ecotypes ($r = 15$ μm).

predominated with the Illinois ecotype (Fig. 4A, B). Spores of *G. mosseae* occurred in such small numbers that statistical comparison was not meaningful.

DISCUSSION

The two populations of *A. gerardii* appear to have adapted to their local environment. Although Kansas and Illinois populations grew larger in the fertile soils of Illinois, each ecotype grew relatively better in its own soil (Fig. 1). Such adaptation to mineral soils has been repeatedly demonstrated (Chapin, 1980), suggesting an underlying trade-off that prevents a single genotype from growing best in soils of high and low fertility. Several mechanisms underlying this trade-off have been suggested, including alterations in root morphology, root : shoot ratio, the production of root exudates, differing nutrient uptake kinetics, and mycorrhizal fungi (Marschner, 1995; Smith and Read, 1997; Lambers, Chapin, and Pons, 1998). Our results suggest that one mechanism enabling the adaptation of these populations to their mineral soils is a shift in plant physiological dependence on AMF. In our study, the Kansas ecotype had greater dependence upon and derived greater benefit from AMF and was thereby more efficient at nutrient acquisition in the low-nutrient Kansas soils (Fig. 1). The Illinois ecotype did not derive as great a benefit from AMF in Kansas soils, but had a more branched root system (Fig. 3A), which was apparently more efficient in the higher nutrient conditions of Illinois soils than Kansas soils (Fig. 1).

We observed a strong trade-off between dependence on AMF and performance in high-nutrient conditions (Fig. 1 and significance of the three-way interaction, Table 2). This trade-off results from the relative efficiencies of two alternative mechanisms for acquiring soil resources: directly through the fine roots vs. through the mycorrhizal symbiosis. With the functional redundancy of these two uptake mechanisms, we would expect plants with fine root systems to be less dependent upon AMF. In fact, a negative correlation between fibrosity of root systems and mycorrhizal dependence has been repeatedly observed between plant species (e.g., St. John, 1980; Hetrick, Kitt, and Wilson, 1988; Bryla and Koide, 1990; Hetrick, Wilson, and Cox, 1992; Hetrick, Wilson, and Todd, 1992). In this study, we demonstrate the same trade-off between ecotypes of the same species. The Kansas ecotype, which had greater mycorrhizal dependence, also had a coarser root system (less branching per unit root length) than the Illinois isolate (Fig. 3A, Table 3).

In adapting to low-nutrient soils, the Kansas ecotype of *A. gerardii* apparently evolved a greater dependence on AMF, perhaps reflecting the increased efficiency of mycorrhizal uptake over direct uptake via roots. In evolving greater dependence on AMF, the Kansas ecotype would also be expected to incur higher costs in supporting the fungus. We found evidence of increased investment by the Kansas ecotype in AMF. We first note that we did not observe significantly greater infected root length or density of external mycelia in the Kansas ecotype compared to the Illinois ecotype (Table 3). The absence of a difference may result from harvesting at the end of the growing season, by which time plants were redirecting resources away from roots and toward flower and fruit production. With flowering, the fungus shifts its resources toward spore production and many of the internal and external hyphal structures are reabsorbed or are inviable (Gazey, Abbott, and Robson, 1992; R. M. Miller and C. V. Rivetta, unpublished data). Therefore, measures of internal and external fungal structures at the end of the growing season may not be an accurate measure of fungal biomass. Greater investment by the Kansas ecotype in AMF was evident in our measure of AMF sporulation, with the Kansas ecotype supporting much greater spore volume of AMF than the Illinois ecotype (Table 3, Fig. 3D).

Our observation of higher spore production in the Kansas ecotype of *A. gerardii* compared to the Illinois ecotype is made stronger by noting that we are comparing measures of total AMF biomass per plant. If investment patterns were constant with plant size, we would expect to see patterns in AMF biomass that mirror the plant biomass patterns. Indeed, the Kansas ecotype has greater infected root length in Kansas soil where the Kansas ecotype was larger, while the Illinois isolate had greater infected root length in Illinois soil where the Illinois isolate was larger (Fig. 3B). However, the spore biovolume patterns run counter to this expected correlation with plant biomass. First, there was significantly greater spore biovolume in the Kansas soil, where the overall plant biomass was much less than in Illinois soil (Table 3, Fig. 3D). Second, sporulation was greater in association with the Kansas ecotype in both soils, even though the Illinois ecotype was much larger in the Illinois soil (Table 3, Fig. 3D). The biomass of AMF relative

to plant biomass strengthens the argument that the Kansas ecotype invests more carbon than the Illinois ecotype in AMF.

Due to the increased benefit from AMF, the mycorrhizal Kansas ecotype was able to obtain a relatively high biomass in the low-fertility Kansas soils. The Kansas ecotype was unable to produce as much biomass as the Illinois ecotype in the higher nutrient soils of Illinois, perhaps because of its high investment in AMF and its coarser root system. In contrast, the Illinois population exhibits a reduced allocation to AMF and a reduced dependence on the association through increased allocation to a branching root system. We suggest that this contributed to the Illinois ecotype's higher biomass when grown in Illinois soils even in the absence of AMF. However, this ecotype was less able to take advantage of mycorrhizal fungi when grown in the low-fertility Kansas soils (Fig. 1).

While the hypothesized mycorrhizal mechanism of adaptation that we outline above is well supported by the observed differences in root architecture and allocation patterns, our experimental design did not eliminate an alternative hypothesis of coadaptation of plant populations with their community of mycorrhizal fungi. We only used AMF isolated from Kansas. Therefore, it is possible that the higher responsiveness of the Kansas ecotype compared to the Illinois ecotype to inoculation is due to greater compatibility of the Kansas plant ecotype to the Kansas fungi, resulting from a process of coadaptation. This alternative hypothesis merits further research. However, our observation of reduced responsiveness of *A. gerardii* from Illinois is consistent with previous results, including studies that used native Illinois inocula (Bentivenga, 1988; Anderson, Hetrick, and Wilson, 1994). This consistency suggests that our observations of reduced mycorrhizal dependence of the Illinois ecotype is not due to the particular mycorrhizal inoculum that we used in this experiment.

The root : shoot ratios were not sensitive to the very different belowground strategies pursued by the two ecotypes. The two ecotypes had similar root : shoot ratios overall, though their root : shoot ratios differed in the two soil types (Table 2). Interestingly, the two ecotypes had lower root : shoot ratios when grown within the soil to which they were adapted (Fig. 2). We can, therefore, suggest that the higher root : shoot ratios of the Illinois ecotype within the Kansas soil and the Kansas ecotype within the Illinois soil reflects nonoptimal allocation to their normal pattern of resource acquisition.

Our initial inocula contained four species of AMF, three of which sporulated by the end of the experiment. Distinct patterns of sporulation were observed between two of these species during the course of the experiment (Table 4); *G. microaggregatum* proliferated in association with the Illinois ecotype and *G. occultum* proliferated in association with the Kansas ecotype (Fig. 4A, B). Sporulation may reflect differences in fungal phenology or differences in fungal community composition. However, sporulation patterns observed in other systems have been found to reflect differences in fungal community composition (Bever et al., 1996). Within the present experiment, it is interesting that the dynamics within the fungal community may have resulted from, or contributed to, the growth and allocation differences between the ecotypes that we observed. The AMF community changes quickly in response to the host (Bever et al., 1996; Table 4) and different AMF species have varying effects on plant growth and allocation (e.g., Streitwolf-Engel et al., 1997; Van der Heijden et al., 1998). Therefore, studies of comparative plant physiology within the ecologically relevant contexts of diverse mycorrhizal fungal communities might benefit from integration with explicit investigations of concurrent AMF community dynamics.

Our results corroborate repeated observations that crop plants adapted to highly fertilized environments have low dependence on AMF, even though their root systems remain infected by these fungi. For example, Hetrick, Wilson, and Cox (1992) documented a decline in mycorrhizal responsiveness when wheat was selected for high yield under high-nutrient growing conditions. Further studies with wheat indicate that responsiveness is an inherited trait rather than a response to individual fungi (Hetrick et al., 1995). We can, therefore, suggest that this reduced dependence on mycorrhizal fungi is an inadvertent, yet predictable, outcome of the adaptation of crop plants to highly fertile conditions. Furthermore, we would expect that an effective program selecting for high yield in low-nutrient, unfertilized conditions would involve, whether purposefully or not, the selection for high dependence on AMF. In fact, such a program may be accelerated by purposeful selection for mycorrhizal responsiveness. In conclusion, this study demonstrated that populations of *A. gerardii* have adapted to variation in soil nutrient levels, and it suggests that this adaptation is mediated by a shift in their dependence on mycorrhizal fungi and their root architecture.

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