Local reduction of mycorrhizal arbuscule frequency in enriched soil microsites

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Increased nutrient availability reduces vesicular–arbuscular mycorrhizal (VAM) associations with plants, but whether increased nutrients in small volumes of soil affect local VAM colonization is not known. In a field experiment we investigated VAM colonization at different times following fertilization of small soil patches. Soil volumes of ~1000 cm^3 were treated with a nutrient solution (enriched patch) or distilled water (control patch) on opposite sides of individual plants of the tussock grass *Agropyron desertorum* and the shrub *Artemisia tridentata*. *Agropyron* had significantly lower ($p = 0.03$) arbuscular infection in the locally enriched patches compared to control patches (32 and 40%, respectively). This reduced arbuscule frequency was apparent at the first sampling (3 days following treatment application) and remained lower in each subsequent sampling (as much as 30% lower than the control patches). *Artemisia* revealed a similar pattern in arbuscule frequency but was not statistically significant. Our results suggest that a plant can locally reduce VAM development, since arbuscule frequency specifically was locally reduced even though vesicle and overall infection was not. Since mycorrhizal infection does not increase, we conclude that increased plant root proliferation and uptake capacity are likely to be more important for the exploitation of temporary nutrient pulses or patches than is increased mycorrhizal activity.

Key words: arbuscule, nutrient exploitation, phosphorus, reduced development, regulation of colonization, soil heterogeneity, vesicular–arbuscular mycorrhizae.

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On sait qu'une augmentation de la disponibilité des nutriments réduit les associations endomycorhiziennes arbusculaires (VAM) avec les plantes, mais on ignore si des augmentations de nutriments dans de petits volumes de sol peuvent affecter localement la colonisation par ces champignons. Dans une expérience conduite sur le terrain, les auteurs ont observé la colonisation endomycorhizienne à différents moments suivant la fertilisation de petites surfaces de sol. Ils ont traité de petits volumes de sols, environ 1000 cm^3, avec une solution nutritive (surface enrichie) ou avec de l'eau distillée (surface témoin) sur les côtés opposés d'une même plante individuelle de l'herbacée *Agropyron desertorum* et de l'arbuste *Artemisia tridentata*. L'*Agropyron* montre une fréquence d'arbuscules significativement plus faible ($p = 0{,}03$) dans les surfaces localement enrichies comparativement aux surfaces témoins (32 et 40%, respectivement). Cette diminution de la fréquence des arbuscules apparait dès le premier échantillonnage (3 j après le traitement) et demeure plus faible à chacun des échantillonnages subséquents (jusqu'à 30% plus faible que dans les surfaces témoins). L'*Artemisia* montre un patron semblable de fréquence des arbuscules, mais la différence n'est pas significative. Les résultats suggèrent qu'une plante peut voir sa formation d'endomycorhizes VA réduite, puisque la formation des arbuscules est localement et spécifiquement réduite bien que la formation des vésicules et la colonisation en général ne le soient pas. Puisque la colonisation mycorhizienne n'augmente pas, les auteurs concluent qu'une augmentation de la prolifération racinaire et de la capacité d'absorption sont probablement plus importantes pour l'exploitation de source de nutriments temporaires ou localisés, qu'une augmentation de l'activité mycorhizienne.

Mots clés : arbuscule, exploitation des nutriments, phosphore, développement réduit, régulation de la colonisation, hétérogénéité du sol, mycorhizes à arbuscules et vésicules.

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Introduction

Colonization of plant roots by vesicular–arbuscular mycorrhizal (VAM) fungi may increase nutrient acquisition from the soil, especially nutrients that are less mobile such as soil phosphate (P) (Nye and Tinker 1977; Allen 1991). When plants are well supplied with nutrients such as P, the colonization frequency and degree of VAM colonization of roots tend to decrease (Menge et al. 1978; Braunberger et al. 1991). This regulation of colonization by host plants may be cost effective, since the plant can acquire adequate quantities of P without expending photosynthate on mycorrhizae (Koide and Elliot 1989; Koide and Schreiner 1992; Bloom et al. 1985).

Nutrients are rarely homogeneously distributed within the rooting zone of individual plants (Charley and West 1975; Jackson and Caldwell 1993). Microsites of nutrient enrichment are assumed to be important to the nutrient status of plants (Chapin 1980) and VAM hyphae can be important in the exploitation of soil heterogeneity (St. John et al. 1983). Development of VAM in roots experiencing localized areas of nutrient-enriched soils may be reduced if the nutrient demand of the whole plant is decreased or if roots in these microsites have greater tissue nutrient concentrations (Koide and Li 1990; Braunberger et al. 1991).

We were interested in the short-term dynamics of exploitation of nutrient patches by two Great Basin species that are mycorrhizal, *Agropyron desertorum* (Fisch. ex Link) Schult. and *Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle. Jackson et al. (1990) showed that roots of these species can increase their physiological uptake capacity for N and P in response to local nutrient-rich patches. Since these species are VAM symbionts and VAM can contribute significantly to nutrient exploitation, we addressed the following question: Can a plant in the field locally regulate mycorrhizal colonization in different parts of its root system in response to a patchy nutrient distribution?

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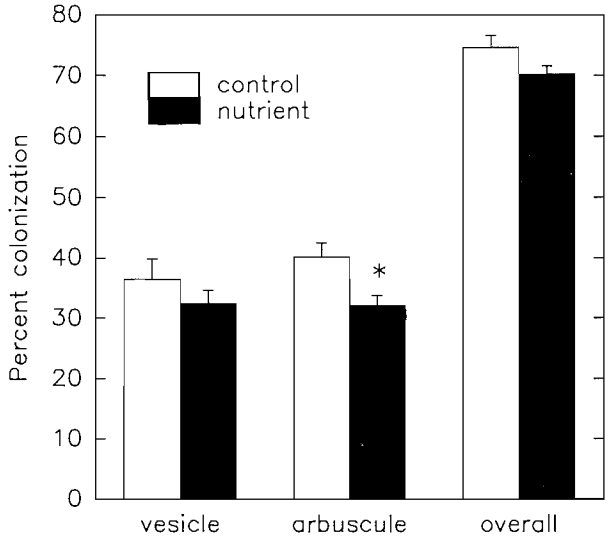

FIG. 1. Percent colonization of vesicles, arbuscules, and overall VAM infection in *Agropyron desertorum*. The vertical bars represent the standard error of each mean ($n = 20$) averaged over date, depth, and treatment. The asterisk indicates statistical significance with $p = 0.03$.

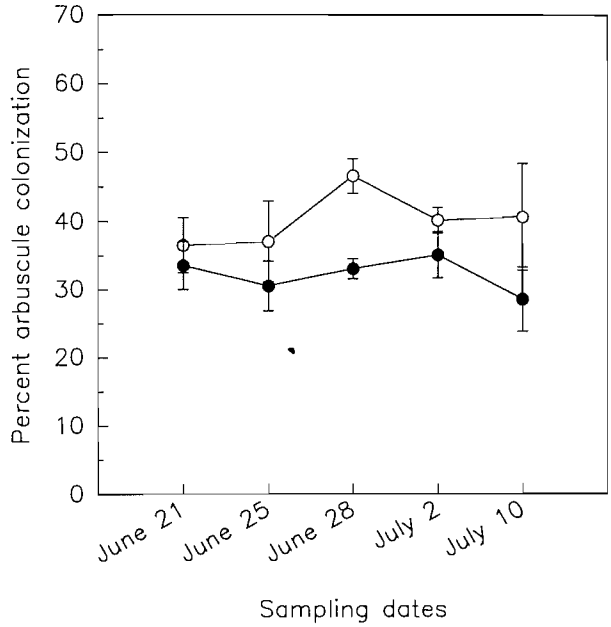

FIG. 2. The percentage of arbuscule colonization of *Agropyron desertorum* at five sampling times for control (○) and nutrient patches (●). The first sampling was 3 days following patch treatment (first split factor). Each mean is averaged over two depths (second split factor) and two plants (main plot factor) (mean ± SE, $n = 4$).

Methods

We conducted a field experiment in 1990, in monoculture stands of two species common to the Great Basin region of the western U.S., the introduced tussock grass *Agropyron desertorum* and the native shrub *Artemisia tridentata*. These plots were established 8 years before the research was conducted at the Green Canyon Research Center. This site is located 4 km northeast of Logan, Utah (41°45′N, 111°48′W, 1460 m elevation). The soil is a calcareous, Typic Haploxeroll, formed from alluvial material (Southard et al. 1978). The average soil pH is ~8.0 and usually contains <10 ppm bicarbonate-exchangeable phosphate (Jackson and Caldwell 1991).

The experiment was conducted in June to determine if a mycorrhizal response to nutrient enrichment changes over several weeks as roots proliferate and to determine if the response varies with soil depth. On June 18, small patches of soil (~1000 cm^3) near the plants were treated with either 300 mL of distilled water or 300 mL of a nutrient solution (20 mM KH_2PO_4, 45 mM NH_4NO_3). In our calcareous soil, this concentrated solution will likely only increase bicarbonate-exchangeable phosphate to ~60 ppm (S.E. Duke, unpublished data; Caldwell et al. 1992) since much of the phosphate is quickly immobilized. Nutrient solutions were introduced evenly into the soil to a depth of 30 cm using pipe cleaners as wicks. On opposite sides of each plant, two control and two nutrient patches were created to reduce some of the natural spatial heterogeneity in the soil. These pairs of control and nutrient patches were within 15 cm of each other and the soil cores from the paired patches were combined at the time of sampling. Within each soil core we investigated two depth ranges: 10–20 and 20–30 cm. We did not sample the 0- to 10-cm depth since fungal hyphae are often not very abundant because of low soil moisture and high temperatures near the soil surface (Allen 1983). Soil cores were harvested, using a 3.5-cm diameter stainless steel corer, 3, 7, 10, 15, and 23 days after treatment application. Two replicate plants for each species were harvested at each of the five sampling dates. Each sample was washed separately using a Hydropneumatic Elutriation Root Washer (Gillison's Variety Fabrication, Inc., Benzonia, Mich.). Organic matter and other soil debris were removed from the samples with forceps. Samples were cleaned within 2 days of collection.

The air-dried roots were stained for mycorrhizal colonization with Trypan Blue using a technique modified from Kormanik et al. (1980). The percent colonization by mycorrhizal fungi was determined from 50 random root segments (1–1.5 cm in length). Colonization was scored for the presence of internal hyphae, vesicles, and arbuscules for each root segment. Overall colonization was defined by the presence of any fungal structure inside the root.

Data were analyzed as a split-split plot ANOVA. The main-plot factor in this design was the plant, split by treatment on two sides of each plant, and each core was then split into two depths. Analysis was performed on each dependent variable (i.e., vesicle frequency, arbuscule frequency, and overall internal infection) separately for each species. Although percentage data are often non-normal, our data were very close to normally distributed and were therefore not transformed prior to analysis.

Results

Arbuscule frequency was significantly reduced for *Agropyron desertorum* roots in enriched soil patches compared with roots in control patches (Fig. 1; Table 1; $p = 0.03$). Roots from nutrient-enriched patches had an average arbuscule colonization of 32%, compared with 40% for roots from control patches. Relative to control patches, arbuscule colonization frequency of *Agropyron* roots in enriched patches was lower for the 3-week time course (Fig. 2). There were no changes in vesicle frequency ($p = 0.43$) and the overall VAM colonization decreased slightly in enriched patches (Fig. 1; $p = 0.07$). Mycorrhizal characteristics were also not differently influenced by nutrient treatment at the two depths evaluated (treatment × depth effect, $p > 0.26$ for all analyses).

There were no significant treatment differences in any mycorrhizal characteristics for *Artemisia tridentata* roots ($p > 0.11$ in each case; mean overall colonization ~70%, data not shown). Although the relative pattern of lower arbuscule frequencies in roots of nutrient patches was similar to that of *Agropyron*, the data are less conclusive for *Artemisia* because the dark color of its roots made it difficult to accurately iden-

TABLE 1. ANOVA table for arbuscule colonization in *Agropyron desertorum*

Source	df	*F*-value	*p*-value
Date	4	0.65	0.6513
Treatment	1	8.47	0.0334*
Depth	1	0.01	0.9434
Date × treatment	4	0.55	0.7115
Date × depth	4	2.84	0.0823
Treatment × depth	1	1.35	0.2715
Date × treatment × depth	4	1.50	0.2751

NOTE: The data were analyzed as a split-split-plot design with date as a fixed factor; individual plants were the main plot factor, nutrient and control treatment were split on each plant, and each soil core taken for each treatment was split into two depths.
*Significant at 0.05 level.

tify arbuscules. The VAM characteristics for *Artemisia* were not significantly different for the two depths evaluated ($p = 0.31$).

Discussion

The process whereby plants reduce or regulate VAM colonization is not well understood (Koide and Schreiner 1992). Braunberger et al. (1991) found that arbuscule development in maize roots was reduced by a general application of phosphate fertilizer and they concluded that increased shoot P concentrations had a significant influence on reducing arbuscule development. In a split-root pot experiment where the two halves of the pot were either mycorrhizal or nonmycorrhizal, Koide and Li (1990) applied equal amounts of P to plants in two treatment schemes: either both halves of the split-root system received 0.31 μg P/mL or the nonmycorrhizal half received 0.62 μg P/mL and the mycorrhizal half received no P. Their results revealed significantly lower development of mycorrhizal colonization (in the mycorrhizal half) for plants receiving the concentrated treatment (0.62 μg P/mL in the nonmycorrhizal half) compared with VAM development in the treatment with equal P delivered to both halves of the root system. They concluded that physiological alterations of the root tissue in response to P availability must be responsible for the regulation of colonization development, since there were no significant differences in shoot nutrient status for the two P treatments. However, the experimental results might not be generally applicable since VAM colonization was low (~12%) in that experiment. Our results do provide some evidence that when a small portion of a root system has greater nutrient availability than the remainder of the root system, plants can reduce the development of arbuscules in these local sites. This localized reduction in arbuscular infection may be due, in part, to significantly greater phosphate concentrations in the roots from enriched patches compared with roots from control patches (Jackson and Caldwell 1992).

Root proliferation is a primary strategy by which plants can more effectively exploit nutrient patches. Root proliferation was likely to be occurring during the 3-week experimental period (Jackson and Caldwell 1989); however, it is not apparent that root growth was more rapid than fungal development, since neither *Agropyron desertorum* or *Artemisia tridentata* showed reduced vesicle formation or overall colonization. We have additional evidence that shows no change in overall mycorrhizal colonization in response to localized nutrient enrichment (within 7 days of treatment) for two *Agropyron* tussock grasses (R.B. Jackson, unpublished data).

Agropyron desertorum and *Artemisia tridentata* both exhibited rapid physiological responses and increased nutrient procurement from nutrient-enriched patches in the soil (e.g., Jackson et al. 1990; Caldwell et al. 1991). Since mycorrhizal colonization does not increase and arbuscule frequency can decrease in enriched soil patches, we conclude that other physiological mechanisms of the plant are more important for the rapid exploitation of enriched soil patches (also see Caldwell et al. 1992). The reduction in arbuscule frequency that we observed in roots from enriched microsites may suggest a local adjustment of resource transfer between the plant and the fungi within those microsites (although not specifically measured in our experiment). Hence, this phenomenon may potentially increase a plant's efficiency of nutrient exploitation (quantity of nutrient acquired per unit carbon expended belowground; sensu Koide and Elliot 1989).

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Allen, M.F. 1983. Formation of vesicular–arbuscular mycorrhizae in *Atriplex gardneri* (Chenopodiaceae): seasonal response in a cold desert. Mycologia, **75**: 773–776.

Allen, M.F. 1991. The ecology of mycorrhizae. Cambridge University Press, New York.

Bloom, A.J., Chapin, F.S., and Mooney, H.A. 1985. Resource limitation in plants — an economic analogy. Annu. Rev. Ecol. Syst. **16**: 363–392.

Braunberger, P.G., Miller, M.H., and Peterson R.L. 1991. Effect of phosphorus nutrition on morphological characteristics on vesicular–arbuscular mycorrhizal colonization of maize. New Phytol. **119**: 107–113.

Caldwell, M.M., Manwaring, J.H., and Jackson, R.B. 1991. Exploitation of phosphate from fertile soil microsites by three Great Basin perennials when in competition. Funct. Ecol. **5**: 757–764.

Caldwell, M.M., Dudley, L.M., and Lilieholm, B. 1992. Soil solution phosphate, root uptake kinetics and nutrient acquisition: implications for a patchy soil environment. Oecologia, **89**: 305–309.

Chapin, F.S. 1980. The mineral nutrition of wild plants. Annu. Rev. Ecol. Syst. **11**: 233–260.

Charley, J.L., and West, N.E. 1975. Plant-induced soil chemical patterns in some shrub-dominated semi-desert ecosystems of Utah. J. Ecol. **63**: 945–964.

Jackson, R.B., and Caldwell, M.M. 1989. The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. Oecologia, **81**: 149–154.

Jackson, R.B., and Caldwell, M.M. 1991. Kinetic responses of *Pseudoroegneria* roots to localized soil enrichment. Plant Soil, **138**: 231–238.

Jackson, R.B., and Caldwell, M.M. 1992. Shading and the capture of localized soil nutrients: nutrient contents, carbohydrates, and root uptake kinetics of a perennial tussuck grass. Oecologia, **91**: 457–462.

Jackson, R.B., and Caldwell, M.M. 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology, **74**: 612–614.

Jackson, R.B., Manwaring, J.H., and Caldwell, M.M. 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature (London), **344**: 58–60.

Koide, R., and Elliot, G. 1989. Cost, benefit and efficiency of the vesicular–arbuscular mycorrhizal symbiosis. Funct. Ecol. **3**: 252–255.

Koide, R., and Li, M. 1990. On host regulation of the vesicular–arbuscular mycorrhizal symbiosis. New Phytol. **114**: 59–64.

Koide, R.T., and Schreiner, R.P. 1992. Regulation of the vesicular–arbuscular mycorrhizal symbiosis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43**: 557–581.

Kormanik, P.P., Bryan, W.C., and Schultz, R.C. 1980. Procedures and equipment for staining large numbers of plant roots for endomycorrhizal assay. Can. J. Microbiol. **26**: 536–538.

Menge, J.A., Steirle, D., Bagyaraj, D.J., Johnson, A., and Leonard, R.T. 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol. **80**: 575–578.

Nye, P.H., and Tinker, P.B. 1977. Solute movement in the soil–root system. University of California Press, Berkeley, Calif.

Southard, A., Wilson, L., and Erickson, A. 1978. Chemical and physical properties of the soils of the Cache Valley area and eastern portions of Box Elder County, Utah. Utah Agricultural Experiment Station Research Report No. 31, Utah State University Press, Logan, Utah.

St. John, T.V., Coleman, D.C., and Reid, C.P.P. 1983. Growth and spatial distribution of nutrient-absorbing organs: selective exploitation of soil heterogeneity. Plant Soil, **71**: 487–493.