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Endomycorrhizal Role for Interspecific Transfer of Phosphorus in a Community of Annual Plants

Abstract. *Phosphorus-32 applied to leaves of* Plantago erecta *in a serpentine annual grassland reached the shoots of about 20 percent of the close neighbors. Vesicular-arbuscular mycorrhizae connect the root systems of neighbors of different species and probably mediate nutrient transfers among them. Spatial patterns of transfer show that taxonomic affinity, distance from donor, and size of recipient do not serve as predictors of transfer and that models of transfer by simple diffusion are not appropriate. No alternative predictor was discovered. The results underscore the importance of belowground interactions in explaining neighbor effects, but the factors controlling nutrient transfer and its consequences for community structure appear complex.*

Despite advances in population and community ecology, little is known about the controls on production or fitness within natural populations and communities of interacting plants. In some cases, simple models of neighbor interactions based on spatial pattern have predicted production patterns rather precisely, but not all attempts to apply simple models have been so effective (*1*). To date, interactions of root systems have been ignored in studies of local controls on production in natural plant communities, even though such interactions are known to affect both growth and long-term community dynamics (*2*, *3*). We report experiments performed to assess the potential role of interconnections among herbaceous plants made by mycorrhizae in shaping community structure and spatial patterns of community production.

Vesicular-arbuscular mycorrhizae are symbioses formed between fungi of the Endogonaceae and plant roots. These mycorrhizae are ubiquitous, develop best under conditions of low soil fertility (particularly low phosphorus), and generally enhance nutrient uptake by the host plant (*4*), though they may be detrimental to young hosts (*5*). Because susceptibility to colonization varies among plant taxa (*6*) and with plant life history (*2*, *5*, *7*), the effects of vesicular-arbuscular mycorrhizae on the nutrient economy of individual plants may structure growth relations and competitive interactions within a plant community. In addition, mycorrhizal plants may participate in a direct form of interaction that is not available to nonmycorrhizal plants. In pot culture, mycorrhizal formation may alter the balance of competition (*8*). Furthermore, a single vesicular-arbuscular fungal hypha can colonize two neighboring plants of the same or different species (*9*), facilitating movement of nutrients from one plant to another (*10*, *11*).

By understanding the dynamics of nutrient transfer among plants that are functionally connected (*11*) by mycorrhizal hyphae, we may be able to predict some of the consequences of mycorrhizae for the structure of a plant community. We examined two aspects of a community having abundant vesicular-arbuscular mycorrhizae: the formation of interplant hyphal connections, and patterns of ^{32}P transfer among plants in the field. We tested the observed patterns of ^{32}P transfer against three possible predictors of nutrient exchange.

The community we examined is a California serpentine grassland dominated by small annual plants (mean density about 1×10^4 m^{-2}). Serpentine soils are characteristically low in most nutrients, including phosphorus (*12*). In preliminary harvests, all species from plant families that typically form mycorrhizae were colonized at the seedling stage by fungi of the genus *Glomus*.

To assess whether the root systems of plants from this grassland may be interconnected by fungal hyphae, a pot experiment was undertaken, and the buried slide technique was used (*13*). Agar-coat-

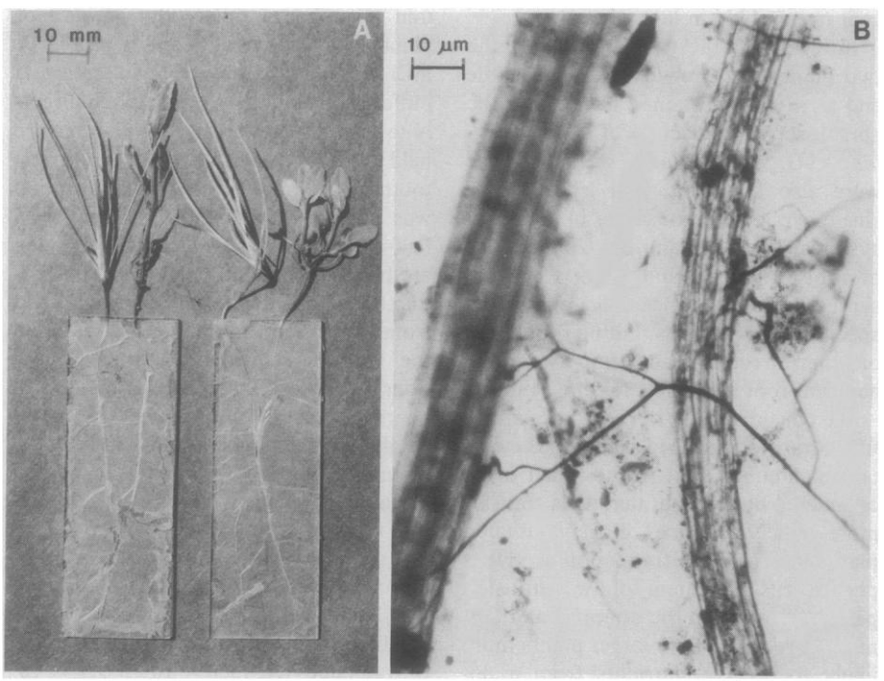

Fig. 1. Roots and mycelium on buried slides. (A) Freshly harvested slides with *Plantago erecta* (left plant on each slide) and *Clarkia rubicunda* (right). (B) At the right, a hypha branches and the right branch enters a *Clarkia* root. The left branch enters a *Plantago* root in several places.

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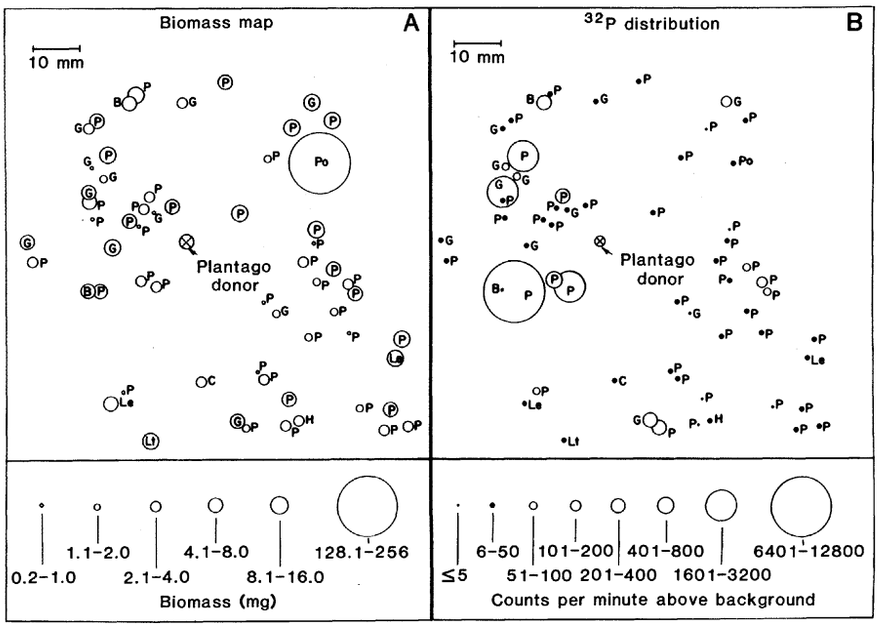

Fig. 2. Schematic maps of one plant neighborhood. (A) Distribution of aboveground biomass (dry weight). (B) Distribution of ^{32}P. Areas of circles approximate relative biomass or counts per minute. P, *Plantago erecta*; C, *Clarkia rubicunda*; B, *Brodiaea pulchella*; H, *Hemizonia luzulifolia*; Le, *Lepidium nitidum*; Lt, *Lotus subpinnatus*; Po, *Poa scabrella*; and G, Gramineae species.

ed glass slides were buried on a slant in serpentine soil. At the higher end, two seeds were planted along with a small inoculum of fresh serpentine soil containing *Glomus* hyphae. One of the seeds was of *Plantago erecta*, the other was of *Clarkia rubicunda* or *P. erecta*. After 2 months, the slides were removed and the agar-embedded roots were cleared in 10 percent KOH and stained with 0.05 percent trypan blue in lactophenol.

In all cases, the root systems of the two plants were readily distinguishable and were deeply stained because of the abundance of arbuscules of *Glomus tenuis* and *G. fasciculatus*. External hyphae were also abundant, and frequently a single hypha had arbuscules in both root systems (Fig. 1, A and B). Connections of roots, hyphae, and roots were observed between conspecific and interspecific pairs of plants, but no root grafts or other direct connections between the adjacent root systems could be found.

We considered three predictors of nutrient transfer between plants in the natural community that we assumed to be connected by hyphae: taxon (Is transfer more likely among closely related plants?); distance from donor (Does phosphorus movement follow a diffusion pattern away from the donor?); and biomass of receptor (Are larger plants more likely to receive nutrients from a donor?). These three factors may be important in determining the number of hyphal connections (or the extent of passive diffusion, though phosphates are highly immobile in the soil) between plants, and in determining their relative sink strengths for a nutrient.

To test these predictors, ^{32}P transfer was mapped at five places within a 0.02-ha area of grassland. In each site, a central plant of *P. erecta* was selected as the "donor." Developing inflorescences, which might provide an important phosphorus sink within the donor plants, were removed and about 0.01 mCi of ^{32}P in 0.1 ml of phosphoric acid buffered to neutrality was applied directly to leaf surfaces. Adjacent plants were held out of contact with the donor by toothpicks. Each entire neighborhood was protected from rain and dew by a half-cylinder of wire mesh that was open at the ends and covered with plastic. Plant neighborhoods were photographed from above, and maps (verified later in the field) were prepared from projections. After 6 or 7 days, donor plants and all neighbors of all species (52 to 88 neighbors per donor) from within 35 to 55 mm of the donor were cut at the soil surface and placed directly in scintillation vials for drying and counting. Čerenkov radiation was counted with a scintillation counter and corrected for decay to day 1 of counting.

All donor plants retained high amounts of radioactivity ($3.1 \times 10^6 \pm 9.7 \times 10^4$ count/min), suggesting that assimilation through the cuticle was inefficient (*14*). Nonetheless, an average of 21 ± 6 percent (range, 4 to 36 percent) of harvested neighbors received significant transfer—more than 50 count/min above background (*15*)—in amounts that spanned a 200-fold range. The recipients included representatives of eight of the 18 taxa (seven families) of neighbors. The remaining ten taxa combined made up less than 5 percent of the harvested neighbors (16 individuals). Only one species, *Lepidium nitidum* (Brassicaceae), the only species we encountered from a family whose members rarely form mycorrhizae, had a frequency of greater than 1 percent without receiving significant transfer. Taxonomic affinity, therefore, does not account for transfer interactions.

Schematic maps for one neighborhood based on dry biomass and on radiation levels (Fig. 2, A and B) show that there is no relation between spatial pattern (distance from the donor) or dominance pattern (biomass of neighbors) and the amount of ^{32}P transfer. The results from all other neighborhoods support the conclusion that transfer was not a function of any of our proposed predictors. For neighbors receiving significant transfer (pooled over all donors, $N = 72$), the best-fit lines from regressions of biomass on counts per minute and distance on counts per minute had slopes that are not significantly different from 0 ($r^2 = .0006$ and .005, respectively). Thus a diffusion model for transfer based on aboveground spatial patterns is inappropriate whether it assumes soil diffusion or direct transfer by mycorrhizal fungi (*16*).

Our results show that ^{32}P moves among neighbors and that hyphae form linkages between root systems, but taxon, aboveground distance, and relative size of the plants are not predictors of the amount of transfer. Although net flow of phosphorus has not yet been measured (because equal amounts of unlabeled phosphorus could have moved in the opposite direction), we expect approximate equilibrium in movement of isotopes after 6 days. If this expectation is reasonable, the high variance in ^{32}P retained by neighbors after that time is indirect evidence that the observed distribution of ^{32}P does reflect net flow (*17*).

The apparent randomness of transfer may in part be due to the time scale of connections. Because fine root branches senesce within weeks and are replaced in function by younger root branches, the pattern of functional connections within a plant neighborhood is likely to change many times during the growth cycle of even ephemeral annual plants.

Vesicular-arbuscular mycorrhizal connections appear to form a network by

which phosphorus (and probably other nutrients) are transferred among higher plants. Although the mechanisms and dynamics of transfer are not yet known, this phenomenon has serious implications for studies of plant microhabitats, interplant competition, comparative demography and phenology, and ecosystem nutrient cycling. Because nutrient exchange potentially contributes to the differential success of individuals, attempts to relate plant success only to aboveground neighborhood structure or spatial pattern (*1*) may prove ultimately unsuccessful.

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11. The mechanism for transfer of nutrients from host to mycobiont is not known [R. E. Beever and D. J. W. Burns, *Adv. Bot. Res.* **8**, 128 (1980)]. Root exudation of phosphorus followed by uptake by adjacent hyphae (root-soil-hypha pathway) has been proposed, but direct transfer from host to mycobiont (root-hypha pathway) is suggested by J. Whittingham and D. J. Read in (*10*). Our term "functionally connected" is intended to emphasize that we are not certain whether root exudation is involved in transfer, but we expect that hyphae will enhance transfer in either case.
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14. In an earlier experiment, an acidic solution of ^{32}P was used, and the cuticular barrier to uptake was breached. Donors retained an average of only 51 percent of the recovered ^{32}P (compared with more than 90 percent when buffered), suggesting that added phosphorus is readily mobile once it gains entry into the plant.
15. Background variation was low (standard error, 2.3 count/min), and there was no evidence of cross-contamination of the samples. We assumed that plants with fewer than 5 count/min (~ 2 standard errors) had received no ^{32}P and, to allow conservatively for errors such as aerial transport and cross-contamination, we assumed that plants with 6 to 50 count/min (up to 22 standard errors) had not received a significant amount of ^{32}P directly through their root systems.
16. Our buried-slide experiments suggest that the number of hyphal connections between roots is related to their proximity. Because there is a correlation (albeit weak) between shoot and root distributions, we do not suspect that the patterns (Fig. 2) result merely from anomalous root distribution but that there is a significant stochastic component to the establishment of the symbiosis relative to aboveground spatial patterns.
17. In a second set of experiments, we tested net flow of phosphorus by excising developing inflorescences, which should constitute strong phosphorus sinks, from half the neighbors of each of five donors. Among plants receiving significant ^{32}P transfer, there was no significant difference between experimental and control plants and no correlation between the specific activities of vegetative and reproductive portions of controls. These results may be explained by the fact that the mycorrhizal symbiosis is dependent on root exudation, which may be altered during the development of inflorescences (J. A. Menge, personal communication).
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Calcium Ionophore A23187 Stimulates Cytokinin-Like Mitosis in *Funaria*

Abstract. *The plant hormone cytokinin stimulates asymmetrical division in target cells of the protonema of the moss* Funaria hygrometrica, *leading to bud formation. The initial division can be induced in the absence of cytokinin by the calcium ionophore A23187 in medium containing calcium. These findings suggest that increases in the concentration of intracellular calcium are essential to bud initiation. Therefore mitotic regulation by cytokinin may be due, at least in part, to the modulation of intracellular calcium ion concentration.*

The plant hormone cytokinin evokes a wide spectrum of responses in higher plants, including the induction of cell division (*1*). In the filamentous protonema of *Funaria hygrometrica* Hedw., exogenous cytokinin induces an asymmetrical division at the distal ends of elongate target cells (caulonema cells and the basal cell of side branches) to form a small initial cell (*2*). This initial cell continues to divide in three planes, leading to bud formation and the leafy gametophyte. Using the fluorescent Ca^{2+} chelate probe chlorotetracycline (CTC), we previously found an increase in membrane-associated calcium at the presumptive bud site in the target cell after cytokinin treatment (*3*). The CTC fluorescence remained bright in the dividing cells of the bud, suggesting that the hormone achieves at least part of its stimulatory effect through a localized modulation of the concentration of intracellular Ca^{2+}. In addition, bud formation is inhibited in Ca^{2+}-free medium and by the Ca^{2+} transport inhibitors La^{3+}, D 600, and verapamil, indicating that cytokinin is dependent on external Ca^{2+} (*4*). We have tested our hypothesis linking cytokinin and Ca^{2+} further, and report that artificially generated increases in intracellular Ca^{2+} caused by the divalent ionophore A23187 (*5*) induce bud initiation in the absence of exogenous cytokinin.

Sterile protonemata were grown from single spores on nutrient agar for 8 to 10 days (*6*). Production of the target caulonemata (which are characterized by small chloroplasts and oblique end walls) is enhanced by transfer to liquid medium for 4 days. Two protonemata (mean fresh weight, 14 mg) were transferred to 15 ml of fresh medium containing 0.3 m*M* Ca^{2+} and 0.1 m*M* Mg^{2+} (*p*H 6.4), and 1 to 60 μl of A23187 stock solution (15 m*M* dissolved in 100 percent methanol) (*7*) was pipetted over the tissue and quickly stirred. Much of the ionophore was visible as a milky precipitate (*8*). Control media included one with equivalent amounts of methanol, one with no additives, and one treated with 1 μ*M* benzyladenine, a synthetic cytokinin. Bud production was monitored for several days (*9*).

Filamentous tip growth and side branch formation continued in both the methanol-treated and no-additive media (Fig. 1A). In protonemata grown in liquid culture initiation of side branches occurred at the seventh or eighth cell from the tip, whereas on agar initiation took place at the third cell. Two successive transfers to fresh media ensured that naturally formed cytokinins did not build up in the medium to cause bud forma-

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