The Ecology and Functioning of Vesicular-Arbuscular Mycorrhizas in Co-Existing Grassland Species. II. Nutrient Uptake and Growth of Vesicular- Arbuscular Mycorrhizal Plants in a Semi-Natural Grassland

I. R. Sanders; A. H. Fitter

New Phytologist, Vol. 120, No. 4 (Apr., 1992), 525-533.

Stable URL:
http://links.jstor.org/sici?sici=0028-646X%28199204%29120%3A4%3C525%3ATEAFOV%3E2.0.CO%3B2-N

New Phytologist is currently published by New Phytologist Trust.

New Phytol. (1992), **120**, 525–533

The ecology and functioning of vesicular–arbuscular mycorrhizas in co-existing grassland species

II. Nutrient uptake and growth of vesicular–arbuscular mycorrhizal plants in a semi-natural grassland

BY I. R. SANDERS* AND A. H. FITTER

Department of Biology, University of York, Heslington, York YO1 5DD, UK

(*Received 25 February 1991; accepted 14 October 1991*)

SUMMARY

Although vesicular–arbuscular mycorrhizas (VAM) are abundant in natural ecosystems, the evidence for the mutualistic effect of this association under field conditions is conflicting. VAM infection and inflow of phosphorus, copper, zinc and manganese was measured in six co-existing plant species of a semi-natural grassland, during a 2-yr period, in order to establish whether patterns of nutrient uptake and plant growth in co-existing species can be related to levels of mycorrhizal infection. There was no relationship between VAM infection and shoot concentrations of P, Cu, Zn or Mn, or plant biomass, and only in a few cases was nutrient inflow related to infection.

Although the results do not support the hypothesis that VAM infection is beneficial for plant nutrition, high levels of P inflow do occur at certain periods during the growth season, suggesting that mycorrhizas may promote plant nutrient uptake at these times. Owing to the abundance of VAM, the lack of speciation and high P inflows which can occur in the field, these mycorrhizal associations are likely to be mutualistic.

Key words: VA mycorrhizas, P inflow, grassland ecology, co-existence.

INTRODUCTION

Vesicular–arbuscular mycorrhizal symbiosis (VAM) can be of great importance in plant nutrition and growth (Harley & Smith, 1983). Evidence to support this largely derives from pot experiments where plants have been grown either with or without mycorrhizal fungi. In such circumstances mycorrhizal infection typically leads to higher root and shoot concentrations of P (for a review see Harley & Smith, 1983). and this in turn leads to an increase in plant growth (Sanders & Tinker, 1973) and biomass (Stribley, Tinker & Rayner, 1980). More precisely the P inflow into plant roots (the rate at which P enters the root from the soil) has been shown to be greater in mycorrhizal roots than in non-mycorrhizal roots (Sanders & Tinker, 1973; Smith, 1982). Many soils are P deficient (Bieleski, 1973) and zones of P depletion rapidly develop in the soil immediately surrounding roots (Bagshaw, Vaidyanathan & Nye, 1972; Nye & Tinker, 1977), because the low mobility of phosphate ions in the soil limits replenishment. Fungal hyphae can cross these zones and transport phosphorus to the roots for a much smaller biomass investment than roots. A mechanism exists, therefore, by which a fungal symbiont can exploit a greater volume of the soil to improve plant P nutrition. In addition, VAM may be important to a lesser extent in increasing the uptake of other immobile nutrients. In particular Cu (Gildon & Tinker, 1983) and zinc (Swaminathan & Verma, 1979). More recently, mycorrhizal infection has been shown to influence the uptake of nutrients which are more mobile in the soil. For example, in mycorrhizal plants the uptake of Mn can be reduced when it occurs at toxic levels (Pacovsky, 1986; McGee, 1987; Arines, Vilarino & Sainz, 1989, 1990).

Few studies of mycorrhizal functioning under field conditions have provided conclusive evidence for a benefit to the host from this relationship. The evidence for a beneficial effect under field conditions is conflicting. For example, Sparling & Tinker (1978)

* Present address: Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA.

recorded no improvement in the yield of upland grassland, following inoculation of γ-irradiated soil, whereas Hayman & Mosse (1979) showed a significant increase in yield, although this could not be explained by increased P uptake.

Since the presence of VAM fungal hyphae in roots must represent a cost to the plant (Koide & Elliott, 1989) it could be argued that the density of infection should be related to the degree of benefit derived by the plant. Studies attempting to relate plant P status with quantitatively different VAM infection levels (Fitter, 1986; McGonigle & Fitter, 1988*a*) are, however, also inconclusive.

Although it is known that VAM infection density varies in space and time in natural ecosystems (Gay, Grubb & Hudson, 1982; Giovannetti, 1985; McGonigle, 1987), little is known about the causes of variation in infection and whether infection is related to plant nutrition and growth. However, if mycorrhizas do benefit plants in the field then periods of high mycorrhizal infection might be expected to correspond with periods of rapid nutrient uptake or high demand for nutrients. Information regarding temporal patterns of VAM infection together with nutrient uptake and plant performance is essential to an understanding of their significance in natural ecosystems. In this study the patterns of VAM infection, mineral nutrition and growth of the major plant species of a semi-natural grassland were recorded over a 2-yr period. In addition, the rates of P, Cu, Zn and Mn uptake were recorded during the growth season.

The aim of the study was to establish whether patterns of nutrient uptake and growth can be directly related to levels of mycorrhizal infection in the field. The specific objectives were (1) to determine whether rates of nutrient uptake in this community were sufficiently high to suggest mycorrhizal involvement, (2) to identify periods in the growth season when mycorrhizal involvement may have been required, (3) to find whether any such periods recurred in successive years, and (4) to establish if interspecific differences occurred in nutrient uptake patterns.

MATERIALS AND METHODS

Field site

This investigation was carried out in conjunction with a survey of patterns of mycorrhizal occurrence at the same field site. A full description of survey methods is given in Sanders & Fitter (1992). All fieldwork was carried out on a permanent grassland site at Wheldrake Ings (North Yorkshire, UK). Owing to the management regime in operation, the community is thought to be as near equilibrium as possible for such a grassland and to contain a relatively undisturbed VAM community. For a fuller site description see McGonigle & Fitter (1988*a*).

Sampling procedure and species selection

Sampling procedures and species selection were as in Sanders & Fitter (1992). Six species (*Festuca rubra* L., *Holcus lanatus* L., *Lathyrus pratensis* L., *Plantago lanceolata* L., *Rumex acetosa* L. and *Trifolium pratense* L.) were sampled on seven occasions (16 February, 29 March, 19 April, 10 and 31 May, 21 June and 21 October) in 1988 and 1989. Plants were removed from the field site using a 6 cm × 16 cm soil corer. The area was cut for hay in late June of each year. The concentration of P, Cu, Zn and Mn in the plants was not measured at the October harvests because the site was being grazed and little of the above-ground plant material remained.

Measurements and mycorrhizal assessment

The roots of sampled individuals were carefully washed in running water to remove soil and other plant roots. Root lengths were measured using a gridline intersection method (Marsh, 1971) and subsequently cleared (10% KOH) and stained (0·01% acid fuchsin) for determination of mycorrhizal infection (Kormanik & McGraw, 1982). Infection was quantified using a line intersect method (× 100 and × 120 magnification) and expressed as a percentage of root length.

For all plants the shoot dry mass and P, Cu, Zn and Mn concentrations were determined. The shoot material was digested in concentrated nitric, sulphuric and perchloric acids (12 : 1 : 1) and refluxed for 1 h. The digests were diluted for spectrophotometric analysis of P concentration (Allen, 1974) and for atomic absorption spectroscopy to determine Cu, Zn and Mn concentrations.

Calculation of nutrient inflows

Since mycorrhizal benefit is believed to derive from the ability of the hyphae to cross P depletion zones around roots, infection should result in an increased rate of P uptake per unit root length (inflow). Where root growth was exponential, P, Cu, Zn and Mn inflows were calculated using equation (1), which assumes root growth to be exponential. However, where no exponential root growth occurred it was assumed to be linear and equation (2) was used. A summary of the analysis of root growth is shown in Table 1. Means of data for each species at all sites within one harvest were used for the calculations of inflow. Values of P inflow are expressed as pmol m^{-1} s^{-1} and Cu, Zn and Mn inflow as fmol m^{-1} s^{-1}.

$$\text{Inflow} = \frac{(C_2 - C_1)(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)(t_2 - t_1)}, \quad (1)$$

$$\text{Inflow} = \frac{C_2 - C_1}{\frac{1}{2}(L_1 + L_2) \times (t_2 - t_1)}, \quad (2)$$

Table 1. *Mean root lengths and F values from one-way analysis of variance of root length and above-ground biomass in 1988 and 1989, using harvest date as a factor (d.f. = 5)*

	Year	Mean root length (cm)	*F* ratio	
			Root length	Biomass
Plantago lanceolata	1988	83·3	n.s.	12·94***
	1989	182·0		24·88***
Rumex acetosa	1988	104·3		27·36***
	1989	157·4	3·67* (1° 9·13**)	14·60***
Trifolium pratense	1988	109·9		11·59***
	1989	146·5	2·86* (4° 6·91*)	5·15**
Holcus lanatus	1988	125·0	4·44* (1° 18·31***)	15·90***
	1989	107·5		7·60***
Festuca rubra	1988	135·6	6·13*** (1° 24·01***)	7·29***
	1989	132·4		1·24 ns
Lathyrus pratensis	1988	138·2		29·68***
	1989	142·0	5·02** (1° 20·07***)	10·85***

The first five orthogonal polynomial terms have been fitted (in parentheses) for root length and *F* values are given for terms at which no further terms or deviations are significant.

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$; n.s., not significant; 1°, linear term; 4°, quartic term.

where C = plant nutrient content, L = root length, t = time, and subscripts 1 and 2 refer to the harvests either side of the interval.

In addition nutrient flux, the mass of nutrient taken up per unit root surface area per time, was calculated by dividing nutrient inflow by the mean root circumference.

Statistical analysis

For statistical purposes, above ground biomass and root length data were transformed to natural logs. To detect the pattern of growth (variation in biomass) and root growth (or variation in root growth) a one-way analysis of variance of root length data was performed. The lowest degree orthogonal polynomials that could adequately be fitted to the root length data were found in order to determine whether root growth was linear, exponential or otherwise.

Correlation coefficients were calculated to determine the relationship between mycorrhizal infection and plant biomass, plant nutrient concentration (of P, Cu, Zn and Mn) or nutrient inflows. The analyses performed are listed below:

(*a*) Correlation of VAM infection with biomass and concentrations and inflows of P, Cu, Zn and Mn for all the species grouped together over the 2 yr and for each separately.

(*b*) As (*a*) but for each species over the 2 yr and for each year separately.

(*c*) Correlation of VAM infection with P, Cu, Zn and Mn inflows over each harvest interval for the 2 yr considered together and for each year individually, irrespective of species.

(*d*) Correlation of VAM infection with the maximum P, Cu, Zn and Mn inflows of each species for the 2 yr together and for each year considered individually.

RESULTS

Root growth

Significant changes in root growth were detected only in *Holcus lanatus* and *Festuca rubra* during 1988 and in *Rumex acetosa*, *Trifolium pratense* and *Lathyrus pratensis* during 1989 (Table 1). With the exception of *T. pratense* the change in root length of these species fitted the linear term. Since the analysis was carried out on logged values the change in root length in these species is exponential and thus validates the use of equation (1) for the calculation of nutrient inflows. The lack of variation in root length may reflect inadequacies in the core sampling for roots, with length underestimated in most cases. Underestimation of root length affects the interpretation of subsequent calculations of % root length infected and nutrient inflow.

Plant nutrient status, growth and mycorrhizal infection

Mean biomass per plant increased in most species as the growing season progressed, though the change was slight in *F. rubra* (Fig. 1, Table 1). Mycorrhizal

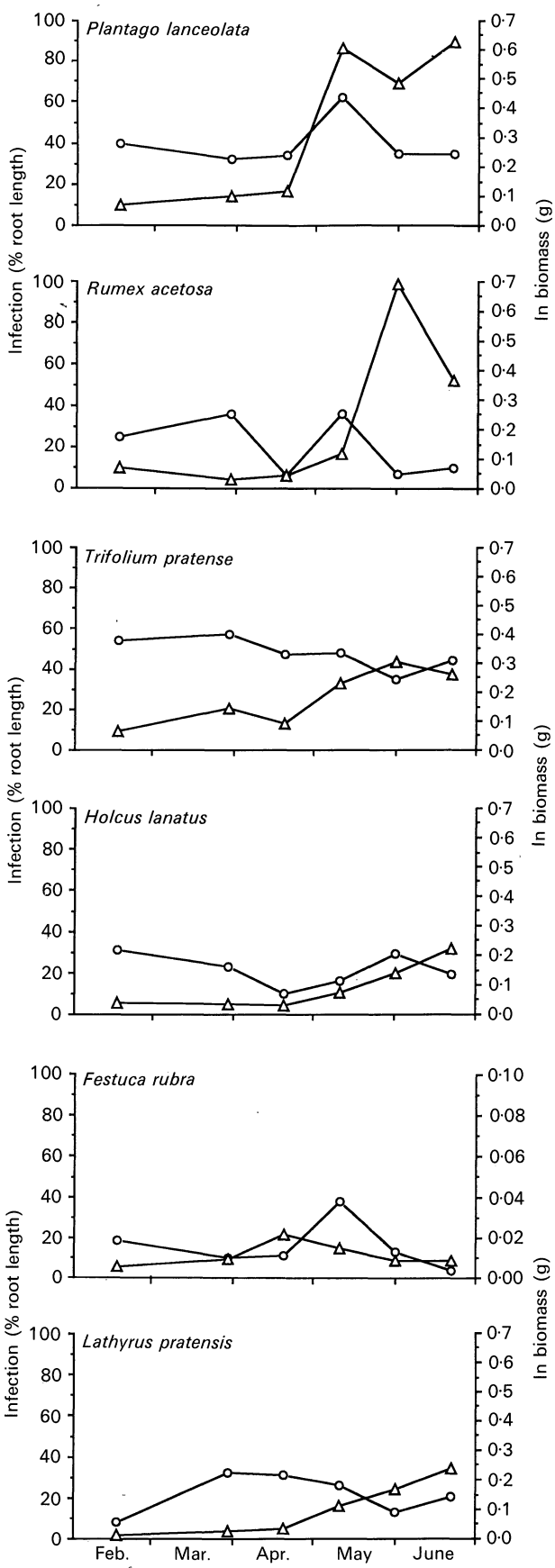

Figure 1. Mean mycorrhizal infection (○) and above-ground biomass (△) in six plant species at Wheldrake Ings during 1989 (note scales for biomass differ).

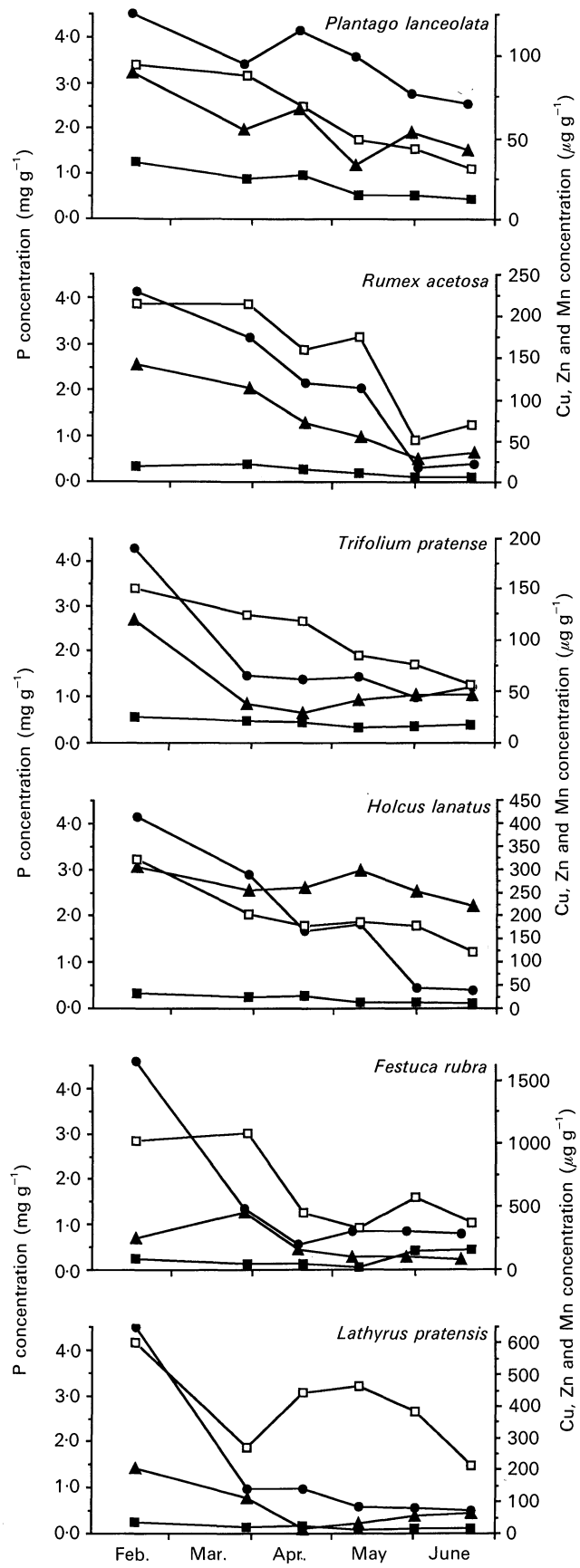

Figure 2. Above-ground mean phosphorus (□), copper (■), zinc (●) and manganese (▲) concentrations in six plant species at Wheldrake Ings during 1989 (note scales differ).

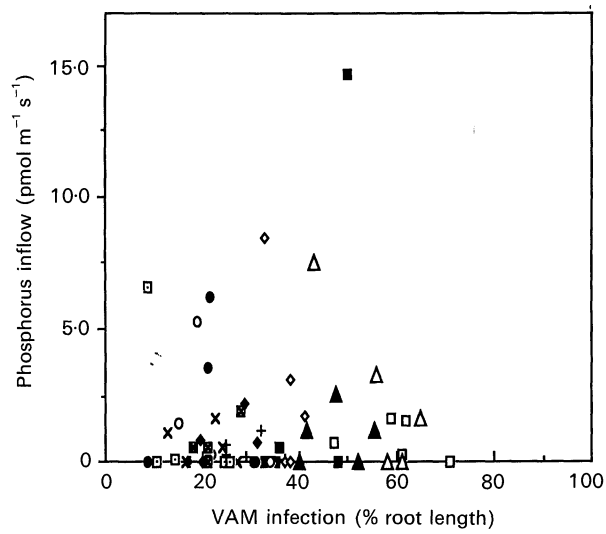

Figure 3. Relationship between phosphorus inflow and VAM infection during 1988 and 1989 in each of six plant species at Wheldrake Ings. *Plantago lanceolata*, 1988 (□), 1989 (■), *Rumex acetosa*, 1988 (○), 1989 (●); *Trifolium pratense*, 1988 (△), 1989 (▲); *Holcus lanatus*, 1988 (+), 1989 (×); *Festuca rubra*, 1988 (■), 1989 (⊡); *Lathyrus pratensis*, 1988 (◇), 1989 (◆).

Table 2. *Significant correlation coefficients of mycorrhizal infection with nutrient inflows*

	n	Correlation coefficient
(*a*) For all species at a given harvest interval		
Phosphorus (interval 1, 1989)	6	0·901*
Copper (interval 4, 1988)	6	0·941**
Manganese (interval 5, 1988)	6	−0·890*
(*b*) Maximum inflow of each species within a year		
Manganese in each species, 1988	6	−0·751*
(*c*) For any one species within a year		
Zinc (in *Trifolium pratense*, 1988)	5	−0·917*

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

infection, however, showed different patterns and average levels between species, the full results of which are presented in Sanders & Fitter (1992).

Nutrient concentrations in above-ground tissues declined in all species during the growing season (Fig. 2). P concentration generally declined progressively, but in *R. acetosa* it fell steeply late in the season, coinciding with a large increase in biomass. Cu concentrations were low at all times ($< 25\ \mu g\ g^{-1}$), whereas Zn ranged from $> 1500\ \mu g\ g^{-1}$ in *F. rubra* in February to $< 25\ \mu g\ g^{-1}$ in *R. acetosa* in June. In all species except *Plantago lanceolata*, Zn concentration declined steeply from February onwards. Mn concentrations were similar in most species (*c.* 100–200 $\mu g\ g^{-1}$) but were high and more or less constant (200–300 $\mu g\ g^{-1}$) in *H. lanatus* and low and declining in *L. pratensis* and *P. lanceolata* (mostly $< 100\ \mu g\ g^{-1}$).

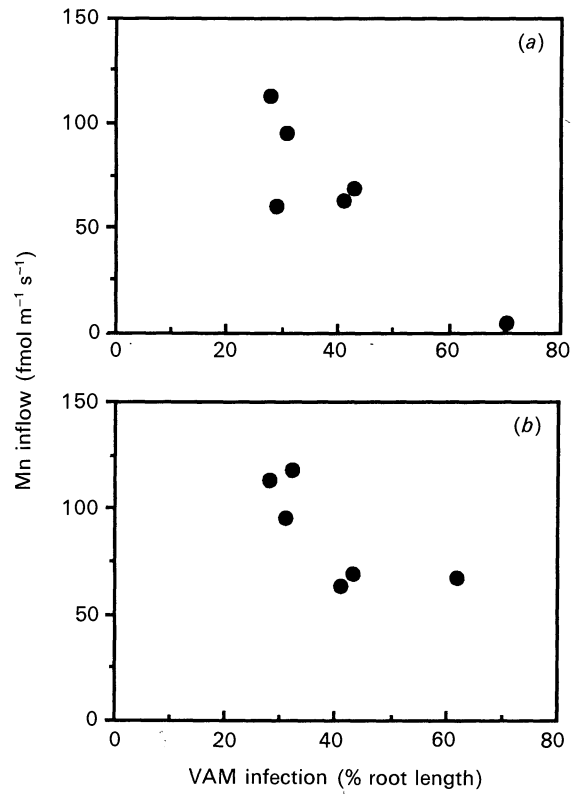

Figure 4. (*a*) Manganese inflow for each of the six species at harvest interval 5 (between 31 May and 21 June) 1988. (*b*) Maximum manganese inflow into each of the six plant species during 1988.

There was no correlation between nutrient concentrations or biomass and VAM infection density.

Rates of nutrient uptake

For most of the study period P inflows were low (between 0·5 and 2·0 pmol $m^{-1}\ s^{-1}$) and only at certain periods in the growth season did P inflows exceed the value of 3·5 pmol $m^{-1}\ s^{-1}$, calculated by Sanders & Tinker (1973) as the maximum 'zero sink' value of P inflow in non-mycorrhizal roots in a similar soil. There was no overall relationship between P inflow and mycorrhizal infection for all species (Fig. 3).

In only 5 out of 231 cases was there a significant correlation between the level of infection and nutrient inflow (Table 2), and the probability that these occurred by chance is high. There were no obvious consistent relationships between mycorrhizal infection and nutrient inflow, except an inverse relationship between VAM infection and Mn inflow for the final harvest in 1988 and the maximum Mn inflows to each species during the same year (Fig. 4*a*, *b*).

Timing of nutrient inflows

In both *P. lanceolata* and *R. acetosa* there were periods of low and of high P inflow (Fig. 5*a*).

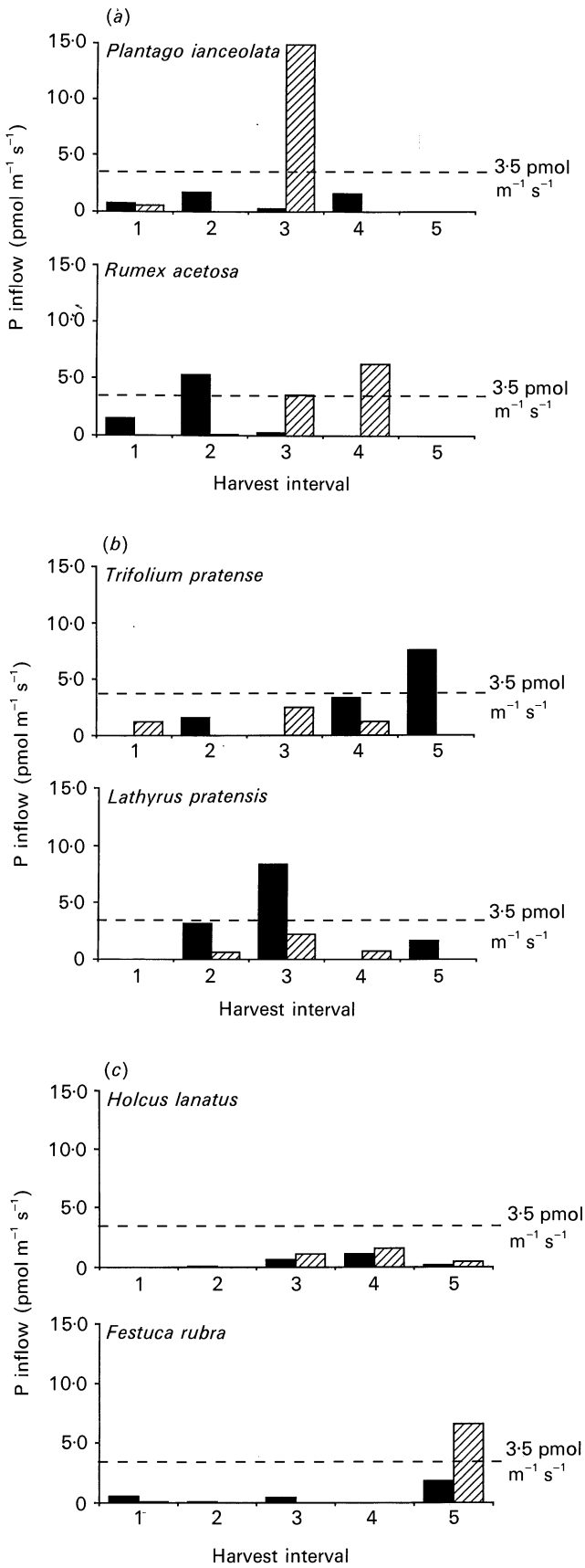

Figure 5. Phosphorus inflow into (*a*) *Plantago lanceolata* and *Rumex acetosa*, (*b*) *Trifolium pratense* and *Lathyrus pratensis*, (*C*), *Holcus lanatus* and *Festuca rubra*, for 5 harvest intervals, where (1) 16 Feb.—29 Mar., (2) 29 Mar.—19 Apr., (3) 19 Apr.—10 May, (4) 10 May–31 May, (5) 31 May—21 June. (■) 1988, (▨) 1989).

Table 3. *Mean root diameter of the six plant species (measured at the 3rd harvest 1989)*

Species	Diameter (cm)	Confidence intervals (95 %)
Plantago lanceolata	0·085	±0·008
Rumex acetosa	0·025	±0·009
Trifolium pratense	0·082	±0·023
Holcus lanatus	0·017	±0·017
Festuca rubra	0·015	±0·006
Lathyrus pratensis	0·035	±0·014

However, the patterns of inflow between the 2 yr were quite different. For example, *P. lanceolata* took up P rapidly over harvest interval three during 1989 (14·8 pmol m^{-1} s^{-1}), although over the same interval during the previous year the rate of P inflow was low (0·3 pmol m^{-1} s^{-1}). *R. acetosa* exhibited its highest rates of P inflow during the first two harvest intervals in 1988. During the same harvest intervals in 1989 P inflow was negligible. *L. pratensis* and *T. pratense* both exhibited periods of rapid P inflow during 1988 which did not recur during 1989 (Fig. 5*b*). Although the rate of P inflow in *L. pratensis* in 1989 did not exceed 3·5 pmol m^{-1} s^{-1}, the overall pattern of inflow during the 2-yr period is similar, with a maximum over harvest interval 3 in each year.

In *F. rubra* and *H. lanatus* periods of high and low P inflow were similar in both years (Fig. 5*c*). *F. rubra* exhibited maximum phosphorus inflow over harvest interval 5 in both years, coinciding with the onset of flowering. In *H. lanatus* maximum P inflow occurred over harvest interval 4 in both years. However, P inflow in *H. lanatus* never exceeded 2·0 pmol m^{-1} s^{-1} during the 2 yr.

Patterns of Cu, Zn and Mn inflow for these species were similar to those for P inflow. Periods of high inflow for *P. lanceolata* and *R. acetosa* in one year coincided with low inflow rates in the following or previous year. The two legumes, *L. pratensis* and *T. pratense*, both exhibited similar patterns of Cu and Mn uptake between the 2 yr. However, there were no clear patterns of Zn uptake in the same two species. In contrast to *P. lanceolata* and *R. acetosa*, the grasses *F. rubra* and *H. lanatus* showed very similar patterns of nutrient uptake during the 2-yr period, i.e. periods of high nutrient uptake in one year recurred in the following year. Inflow data for all species are given in Sanders (1991).

Interspecific differences in rates of nutrient uptake

There were differences in the pattern of nutrient uptake between years for any one species and also between the six species (Fig. 5*a–c*). However, because of large differences in root diameter between the six species (Table 3), comparisons of inflows may

Table 4. *Maximum phosphorus inflows (pmol m^{-1} s^{-1}) and fluxes (nmol m^{-2} s^{-1}) into roots of the six plant species during 1988 and 1989*

Species	Year	P inflow	P flux
Plantago lanceolata	1988	1·7	0·6
	1989	14·8	5·6
Rumex acetosa	1988	5·3	6·7
	1989	6·2	7·9
Trifolium pratense	1988	7·5	2·8
	1989	2·5	1·0
Holcus lanatus	1988	1·1	2·1
	1989	1·6	3·0
Festuca rubra	1988	1·9	4·0
	1989	6·6	14.0
Lathyrus pratensis	1988	8·4	7·6
	1989	2·2	2·0

be misleading. For an interspecific comparison of nutrient uptake rates an estimation of nutrient fluxes (uptake per unit surface area per unit time) were calculated.

The maximum P flux into each of the six species (Table 4) indicated that the rates of P movement differed between species. For example, *P. lanceolata* exhibited the maximum P inflow, although the P flux for the same harvest was greatly exceeded by that of *F. rubra*, *L. pratensis* and *R. acetosa* at other harvests (maximum values of flux were 14·0, 7·6 and 7·9 nmol m^{-2} s^{-1} respectively). After conversion to flux the continuously low P inflow which occurred in *H. lanatus* was comparable to other species, e.g. *T. pratense*, which exhibited high P inflow at some harvest intervals. Interspecific differences in rates of Cu, Zn and Mn movement also occurred (Table 5). A full list of flux values for P, Cu, Zn and Mn are given in Sanders (1991).

DISCUSSION

The relationship between VAM infection and nutrient uptake

The results of this investigation give little support to the hypothesis that there is a relationship between the density of VAM infection and nutritional benefits to plants in natural ecosystems. The inverse relationship between VAM infection and Mn inflow over the final harvest interval in 1988 and maximum inflows in each species during the same year (Fig. 4) was consistent with experiments carried out under controlled conditions in pots (Arines *et al.*, 1990). The relationship did not recur during 1989, suggesting an environmental influence; Mn will be more mobile during periods of high soil water content, and Wheldrake Ings is subjected to periods of flooding which vary between years (McGonigle & Fitter, 1988*b*). Although soil moisture was not measured in this study the flood level was higher during 1988 than in 1989 and persisted until later in the spring. Further, 1988 was a wet, and 1989 an exceptionally dry, summer.

Although relationships between P uptake and root length infected by VAM fungi have been found under controlled conditions, these may be modified in natural ecosystems. Plant P uptake mediated by mycorrhizal fungi must depend on (1) how much of the soil can be exploited by the external hyphae, (2) the rate at which these hyphae take up the available P and (3) how much of the internal fungus is active in transferring P to the plant. None of these factors are well documented in field soils, and all will be dependent on numerous variables which can be stabilised under carefully controlled laboratory conditions.

Furthermore, in the field, once some threshold density of external VAM hyphae is exceeded, P could be as limiting to the VAM hyphae as to the

Table 5. *Maximum copper, zinc and manganese inflows (fmol m^{-1} s^{-1}) and fluxes (pmol m^{-2} s^{-1}) into roots of the six plant species during 1988 and 1989*

		Maximum inflow			Maximum flux		
Species	Year	Cu	Zn	Mn	Cu	Zn	Mn
Plantago lanceolata	1988	16·3	15·5	67.4	6·1	5·8	25·3
	1989	64·7	426.0	140.4	24·2	159·5	52·6
Rumex acetosa	1988	12·7	71·5	95·6	16·2	91·0	121·8
	1989	38·6	61·2	241·3	49·2	77·9	307·2
Trifolium pratense	1988	19·8	21·2	57·9	7·5	8·0	22·0
	1989	12·0	57·9	54·2	4·6	22·0	20·5
Holcus lanatus	1988	7·2	28·9	118·0	13·5	54·1	221·0
	1989	7·8	54·9	185·5	14·6	102·7	347·3
Festuca rubra	1988	43·9	157·6	113·2	93·3	334·4	240·2
	1989	21·2	134·4	96·7	45·0	285·3	205·5
Lathyrus pratensis	1988	24·9	62·6	63·3	22·7	56·9	57·6
	1989	8·8	40·1	59·0	8·0	36·4	53·7

roots, when the combined hyphal and root density is so high that the rhizosphere and mycorrhizosphere become wholly depleted of P. In a closely compacted turf like that at Wheldrake Ings, with high mycorrhizal infection and high root density (over 100 cm root cm^{-3} soil, McGonigle & Fitter, 1988*b*), rapid growth of the hyphae may not necessarily enable the fungus to exploit new sources of P which are unavailable to the root.

Implications for VAM functioning and ecology

Possible reasons as to why direct relationships between mycorrhizal infection and plant nutrition and growth are difficult to identify in natural conditions are numerous and well documented (Fitter, 1985, 1990). At the field site it is reasonable to assume that some phenomena limit mycorrhizal benefit, although their nature and extent is difficult to determine. Plants with low mycorrhizal infection could receive nutrients via inter-connecting hyphae from other plants of the same or a different species (Newman, 1988), removing any relationship between internal VAM infection and plant nutrient uptake. In addition, the length of root occupied by VAM fungi does not necessarily represent the amount of fungus which is active in nutrient transfer (Kough & Gianinazzi-Pearson, 1986; Abdel-Fattah, 1990; Smith, McGee & Smith, 1990; Smith & Gianinazzi-Pearson, 1990). Grazing by soil fauna can also affect the functioning of VAM (Fitter & Sanders, 1992).

The simplest explanations for the lack of any clear relationship between VAM infection and plant nutrition are either that the fungus is acting as a parasite, i.e. giving no benefit to the host, or that only during periods of high P demand do VAM fungi contribute to the necessary rate of uptake.

Periods of high P demand are likely to be associated with periods of high respiration and photosynthetic rates such as during flowering or seed production, and this is when mycorrhizas may be involved. If this is the case then periods of rapid P uptake, such as those observed in this study (Fig. 5), should coincide with these stages and should recur at the same time in subsequent years. For *H. lanatus* and *F. rubra*, this was the case in this study. *H. lanatus* exhibited maximum phosphorus inflow just before the usual flowering period for this species, as found by McGonigle & Fitter (1988*b*) at the same field site. In contrast, species such as *P. lanceolata* and *R. acetosa* exhibited highly irregular patterns of nutrient uptake which cannot be attributed to a specific period in the growth season.

From the results of this study it is impossible to establish exactly when nutrients were taken up from soil (whether by roots or by VAM fungi), because plant nutrient content was measured in shoots only, the roots having been used for mycorrhizal assessment. Thus, it is possible that the timing of nutrient uptake into the roots could occur some time before transfer to the shoots. This increases the difficulty in detecting a relationship between infection levels and nutrient uptake.

The relationship between infection density and P inflow might be further obscured by the presence of several VAM fungal species, possibly jointly infecting a single root system (McGonigle, 1987; Rosendahl, Rosendahl & Sochting, 1990; Sanders, 1990; Sanders & Fitter, 1992). If VAM fungal species differ in P uptake rates, then variation in P inflow to plants could be due to changes in the relative abundance of VAM fungal species. Some of these species may give little nutritional benefit to the plant. Theoretical support for the persistence of non-functional infections is provided by cost–benefit analysis of VAM infection (Fitter, 1991). Evidence for this also exists from field investigations. McGonigle (1987) showed that the relative abundance of coarse and fine endophytes in the roots of *H. lanatus* changes over time, the fine endophyte probably being *Glomus tenue*, which rarely produces a growth response in the host (Powell, 1979).

Until more field-based investigations quantify VAM infection in terms of infection units and more knowledge is gained on the occurrence and efficiency of different fungal types little progress can be made to establish the role of VAM in natural communities. However, the fact that mycorrhizal plants apparently exhibit low P uptake rates during most of the growth season, with occasional periods of rapid uptake, suggests that mycorrhizas play an important role in plant nutrition at certain times of the year. At present, explanations of the functioning of VAM under natural conditions remains speculative owing to the failure to relate P uptake to functional measures of VAM infection in the field.

ACKNOWLEDGEMENTS

The authors thank the Yorkshire Wildlife Trust and the Nature Conservancy Council for permission to work at Wheldrake Ings. This work was carried out while I.R.S. was in receipt of a studentship from the Natural Environment Research Council, whose support is gratefully acknowledged.

REFERENCES

Abdel-Fattah, G. M. (1990). *Some ecological and physiological studies on vesicular–arbuscular (VA) mycorrhizal fungi.* PhD Thesis, Mansoura University, Egypt.

Allen, S. E. (1974). *Chemical Analysis of Ecological Materials.* Blackwell Scientific Publications, Oxford.

Arines, J., Vilarino, A. & Sainz, M. (1989). Effect of different inocula of vesicular–arbuscular mycorrhizal fungi on manganese content and concentration in red clover (*Trifolium pratense* L.) plants. *New Phytologist* **112**, 215–219.

Arines, J., Vilarino, A. & Sainz, M. (1990). Effect of vesicular–arbuscular mycorrhizal fungi on manganese uptake by red clover. *Agriculture, Ecosystems and Environment* **29**, 1–4.

Bagshaw, R., Vaidyanathan, L. V. & Nye, P. H. (1972). The

supply of nutrient ions by diffusion to plant roots in the soil. V. Direct determination of labile phosphate concentration gradients in a sandy soil induced by plant uptake. *Plant and Soil* **7**, 617–626.

BIELESKI, R. L. (1973). Phosphate pools, transport and availability. *Annual Review of Plant Physiology* **24**, 225–252.

FITTER, A. H. (1985). Functioning of vesicular–arbuscular mycorrhizas under field conditions. *New Phytologist* **99**, 257–265.

FITTER, A. H. (1986). Effect of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. *New Phytologist* **103**, 767–776.

FITTER, A. H. (1990). The role and ecological significance of vesicular–arbuscular mycorrhizas in temperate ecosystems. *Agriculture, Ecosystems and Environment* **29**, 137–151.

FITTER, A. H. (1991). Costs and benefits of mycorrhizas: implications for functioning under natural conditions. *Experientia* **47**, 350–355.

FITTER, A. H. & SANDERS, I. R. (1992). Interactions of mycorrhizas with soil animals. In: *Mycorrhizal Functioning* (Ed. by M. F. Allen). Chapman & Hall, New York (in the press).

GAY, P. E., GRUBB, P. J. & HUDSON, H. J. (1982). Seasonal changes in the concentrations of nitrogen, phosphorus and potassium, and in the density of mycorrhiza, in biennial and matrix forming perennial species of closed chalkland turf. *Journal of Ecology* **70**, 571–593.

GILDON, A. & TINKER, P. B. (1983). Interactions of vesicular–arbuscular infections and heavy metals in plants. II. The effects of infection on the uptake of copper. *New Phytologist* **95**, 263–268.

GIOVANNETTI, M. (1985). Seasonal variations of vesicular–arbuscular mycorrhizas and Endogonaceous spores in a maritime sand dune. *Transactions of the British Mycological Society* **84**, 679–684.

HARLEY, J. L. & SMITH, S. E. (1983). *Mycorrhizal Symbiosis*. Academic Press, Oxford.

HAYMAN, D. S. & MOSSE, B. (1979). Improved growth of white clover in hill grasslands by mycorrhizal inoculation. *Annals of Applied Biology* **93**, 141–148.

KOIDE, R. & ELLIOTT, G. (1989). Cost, benefit and efficiency of the vesicular–arbuscular mycorrhizal symbiosis. *Functional Ecology* **3**, 252–255.

KORMANIK, P. P. & MCGRAW, A. C. (1982). Quantification of vesicular–arbuscular mycorrhizae in plant roots. In: *Methods and Principles of Mycorrhizal Research* (Ed. By N. C. Schenk), pp. 37–46. The American Phytopathological Society, St Paul, Minnesota.

KOUGH, J. L. & GIANINAZZI-PEARSON, V. (1986). Physiological aspects of VA mycorrhizal hyphae in root tissue and soil. In: *Physiological and Genetical Aspects of Mycorrhizae* (Ed. by V. Gianinazzi-Pearson & S. Gianinazzi), pp. 223–226. INRA, Paris.

MARSH, B. (1971). Measurement of length in random arrangements of lines. *Journal of Applied Ecology* **8**, 265–267.

MCGEE, P. A. (1987). Alteration of growth of *Solanum opacum* and *Plantago drumondii* and inhibition of regrowth of hyphae of vesicular–arbuscular mycorrhizal fungi from dried root pieces by manganese. *Plant and Soil* **101**, 227–233.

MCGONIGLE, T. P. (1987). *Vesicular–arbuscular mycorrhizas and plant performance in a semi-natural grassland*. DPhil Thesis, University of York, York, U.K.

MCGONIGLE, T. P. & FITTER, A. H. (1988*a*). Growth and phosphorus inflows of *Trifolium repens* L. with a range of indigenous vesicular–arbuscular mycorrhizal infection levels under field conditions. *New Phytologist* **108**, 59–65.

MCGONIGLE, T. P. & FITTER, A. H. (1988*b*). Ecological consequences of arthropod grazing on VA mycorrhizal fungi. *Proceedings of the Royal Society of Edinburgh* **94B**, 25–32.

NEWMAN, E. I. (188). Mycorrhizal links between plants: Their functioning and ecological significance. *Advances in Ecological Research* **18**, 243–270.

NYE, P. H. & TINKER, P. B. (1977). *Solute Movement in the Spoil–root System*. Studies in Ecology 4, Blackwell Scientific Publications, Oxford.

PACOVSKY, R. S. (1986). Micronutrient uptake and distribution in mycorrhizal and phosphorus fertilized soybeans. *Plant and Soil* **95**, 379–388.

POWELL, C. L. (1979). Inoculation of white clover and ryegrass seed with mycorrhizal fungi. *New Phytologist* **83**, 81–85.

ROSENDAHL, S., ROSENDAHL, C. L. & Sochting, U. (1990). Distribution of VA mycorrhizal endophytes amongst plants from a Danish grassland community. *Agriculture, Ecosystems and Environments* **29**, 329–336.

SANDERS, F. E. & TINKER, P. B. (1973). Phosphate flow into mycorrhizal roots. *Pesticide Science* **4**, 385–395.

SANDERS, I. R. (1990). Seasonal patterns of vesicular–arbuscular mycorrhizal occurrence in grasslands. *Symbiosis* **9**, 315–320.

SANDERS, I. R. (1991). *Seasonality, selectivity and specificity of vesicular–arbuscular mycorrhizas in grasslands*. DPhil Thesis, University of York, Heslington, York, UK.

SANDERS, I. R. & FITTER, A. H. (1992). The ecology and functioning of vesicular–arbuscular mycorrhizas in co-existing grassland species. I. Seasonal patterns of mycorrhizal occurrence and morphology. *New Phytologist* **120** (in the press).

SMITH, S. E. (1982). Inflow of phosphate into mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different levels of soil phosphate. *New Phytologist* **90**, 293–303.

SMITH, S. E., MCGEE, P. A. & SMITH, F. A. (1990). Physiological interactions between mycorrhizal fungi and host plants: an approach to determining the bases of symbiotic efficiency. In: *Endocytobiology IV*, pp. 91–98. INRA, Paris, France.

SMITH, S. E. & GIANINAZZI-PEARSON, V. (1990). Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: effects of photon irradiance and phosphate nutrition. *Australian Journal of Plant Physiology* **17**, 177–188.

SPARLING, G. P. & TINKER, P. B. (1978). Mycorrhizal infection in Pennine grassland. I. Levels of infection in the field. II: effects of mycorrhizal infection on the growth of some upland grasses on gamma-irradiated soil. *Journal of Applied Ecology* **15**, 943–964.

STRIBLEY, D. P., TINKER, P. B. & RAYNER, J. (1980). Relation of internal phosphorus concentration and plant weight in plants infected by vesicular-arbuscular mycorrhizas. *New Phytologist* **86**, 261–266.

SWAMINATHAN, K. & VERMA, B. C. (1979). Responses of three crop species to vesicular-arbuscular mycorrhizal infection on zinc deficient Indian soils. *New Phytologist* **82**, 481–488.