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I. R. Sanders; A. H. Fitter

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

I. Seasonal patterns of mycorrhizal occurrence and morphology.

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

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

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SUMMARY

Vesicular–arbuscular mycorrhizal infection and morphology was measured in six co-existing plant species of a semi-natural grassland during a two year period, in order (1) to measure within-year variation in mycorrhizal infection, (2) to determine whether seasonal trends in mycorrhizal infection were the same in both years, and (3) to quantify and interspecific differences in this variation. Seasonal trends in total mycorrhizal infection in the six plant species grouped together were very different in the two years. When the plant species were considered individually distinct patterns emerged. Mycorrhizal infection in *Plantago lanceolata* L. and *Rumex acetosa* L. varied greatly both within and between years. In contrast, *Festuca rubra* L., *Holcus lanatus* L., *Lathyrus pratensis* L. and *Trifolium pratense* L. all exhibited more constant levels of infection within one year and between successive years. However, interspecific differences in infection occurred between these four species. Although there was temporal variation in hyphal density, arbuscule and vesicle density varied very little with time and thus could not be used to indicate whether there are predictable periods of host cost or benefit. Vesicle width varied significantly between the plant species; this might represent infection by different fungal species.

Key words: VA mycorrhizas, Grassland ecology, VAM morphology.

INTRODUCTION

Vesicular–arbuscular mycorrhizas (VAM) are abundant in temperate ecosystems (Harley & Smith, 1983; Harley & Harley, 1987). This prevalence of VAM in the field has been one of the main pieces of evidence in the argument that they are generally beneficial to plants (Fitter, 1990). If mycorrhizal infection is high in a large number of individual plants in a community, then potentially, a significant part of the photosynthate produced by those individuals could be directed to the fungal symbionts, i.e. the carbon cost to the hosts would be high. However, in a situation of high cost to the host, selection pressure should act to reduce infection, unless there are corresponding benefits, thus decreasing the level of infection within the community (Fitter, 1991).

Knowledge of the seasonal pattern of infection is necessary to quantify the functioning and ecological significance of VAM. Periods during which mycorrhizal infection is high are those when the fungus is most likely to influence plant nutrient status and exert a demand for carbon from the plant. If periods within the growth season exist when numbers of arbuscules are high, this could be a time when nutritional benefits to the host occur. Similarly, when a rapid increase in the abundance of hyphae and vesicles occurs in the root, this could be a period in which the fungus acts as a significant carbon sink.

There is little information about spatial and temporal patterns of VAM infection and although there have been a few studies in a variety of ecosystems, they show no clear pattern. McGonigle (1987) found little temporal variation in the total fractional infection of species in a species-rich grassland, although large differences in infection between species were observed. He also found VAM to be most abundant in roots at a depth of 3–6 cm in the soil. Similarly, in a survey of VAM infection in a temperate deciduous woodland (Brundrett &

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

Kendrick, 1988, 1990*a*) there was 'no marked seasonal pattern in overall infection levels, and variation in infection levels was attributed to periods of root growth and senescence. Sparling & Tinker (1975) also found no significant seasonal change in VAM infection in an upland grassland.

In contrast, some studies have revealed patterns of VAM infection. In three damp meadow or pasture sites in USA, over a wide range of soil pH, the highest colonization of mycorrhizas was recorded in the spring (Rabatin, 1979), a period when root growth would be expected. Marked seasonal patterns of VAM infection have also been recorded in plants of a chalk grassland (Gay, Grubb & Hudson, 1982), where three biennial species were found to exhibit one seasonal trend whilst three perennial species exhibited a different seasonal trend.

However, these studies only monitored the levels of infection for a single year and can therefore only show trends in infection within that period. To demonstrate true seasonal patterns it is necessary to follow infection levels over more than 1 yr.

This investigation involved measuring VAM infection and morphology in six plant species over a period of two years in order (1) to determine whether seasonal trends in VAM infection recurred, resulting in seasonal patterns of VAM infection, (2) to measure within-year variation in VAM infection and (3) to quantify any interspecific differences in this variation. In this paper, changes in level of infection occurring within one year are described as 'trends', whilst consistent trends, i.e. those repeated in consecutive years, are described as patterns.

MATERIALS AND METHODS

Field site

All fieldwork was carried out at Wheldrake Ings, a species-rich, alluvial meadow in Yorkshire, England (national grid reference SE. 702443). The management regime in operation at Wheldrake Ings has remained unchanged for centuries (cut for hay in July and the aftermath grazed by sheep) and consequently the composition of the grassland communities are assumed to be near equilibrium. The clay loam soil (pH 6·0 at a depth of 3–6 cm) contains 41, 16 and 9 mg kg^{-1} dry mass of $NaHCO_3$-extractable phosphorus at depths 0–3, 3–6 and 6–9 cm, respectively (McGonigle & Fitter, 1988).

Sampling procedure and species selection

At seven harvest intervals in each of two years (1988, 1989), individuals of six plant species were removed from eight randomly selected points in a 10×100 m area. The nearest individual of each species, within 1 m of the selected point, was removed using a 6 cm diameter $\times$ 16 cm depth soil corer. It is impossible to assess the proportion of the root system recovered with this method, although it is certain that a proportion of the lateral roots were excluded. Six of the main plant species of the grassland, identified by biomass measurement (Fitter, 1986), were selected for sampling. These were *Festuca rubra* L., *Holcus lanatus* L., *Lathyrus pratensis* L., *Plantago lanceolata* L., *Rumex acetosa* L. and *Trifolium pratense* L. This list comprises two grasses, two legumes and two other dicots, and includes species which are known normally to be heavily infected by VAM, e.g. *P. lanceolata* (Newman, Heap & Lawley, 1981; Eissenstat & Newman, 1990) and one species, *R. acetosa* which has been recorded as non-mycorrhizal (Harley & Harley, 1987).

Harvests were taken on 16 February, 29 March, 19 April, 10 May, 31 May, 21 June and 21 October in both 1988 and 1989. The area was cut for hay in late June of each year.

Measurements and mycorrhizal assessment

The roots of the six species were carefully washed in running water to remove soil and fragments of other plant roots and subsequently cleared in 10% KOH and stained (0·01% acid fuchsin) to determine mycorrhizal infection (Kormanik & McGraw, 1982). Infection was quantified using a line intersect method ($\times 100$ and $\times 120$ magnification) and expressed as a percentage of root length (McGonigle *et al.*, 1990). In addition, when quantifying infection at the first six harvests in 1989, at each intersection the presence or absence of hyphae (H), arbuscules (A), vesicles (V), hyphae and arbuscules (HA), hyphae and vesicles (HV) and hyphae, arbuscules and vesicles (HAV) were recorded and expressed as a percentage of root length. Mean vesicle dimensions (width and length) and internal hyphal diameter were also measured.

Statistical analysis

The mycorrhizal infection and morphological data were expressed as percentages and arcsin square-root-transformed for statistical analysis (Snedecor & Cochrane, 1967). Analyses of variance on total mycorrhizal infection data were carried out to investigate patterns of mycorrhizal infection; the design assumed cross-classification of three factors (species, harvests and years). The hypothesis that patterns of VAM infection are seasonal, i.e. that the same levels of VAM infection occur at the same time in each year, was tested using threeway and twoway ANOVA for the whole data set (all species) and for each of the six species respectively. The nature of within-year variation in infection was examined for each species by a one-way ANOVA and seasonal variation was detected using the lowest degree orthogonal polynomials (i.e. linear, quadratic etc.)

that adequately fitted the data. This was done for 1988 and 1989 separately. In order to analyse the morphological data three new variables were calculated:

$$\text{Hyphae} = H + HA + HV + HAV;$$
$$\text{Arbuscules} = A + HA + HAV;$$
$$\text{Vesicles} = V + HV + HAV,$$

and these were arcsin square-root-transformed before analysis. Because data on the morphology of VAM infection was collected during one year only, a three-way ANOVA was not performed on this data set. The 95% confidence intervals were calculated using the transformed infection data.

RESULTS

Recurring patterns of mycorrhizal infection

Three-way analysis of variance of the complete data set (Table 1) indicated that there were significant differences in the mean levels of infection between species, between years and between harvests. In addition the harvest × year interaction and the second-order interaction were both highly significant. In contrast, the species × year interaction was not significant and the species × harvest interaction was weak.

The existence of some seasonal pattern is indicated by the significant harvest effect. The overall levels of infection, averaged for all six species, were similar for much of the two years. except in April and June when infection was lower in 1989 than in 1988 and in October, when infection in 1989 significantly exceeded that in 1988 (Fig. 1). The highly significant harvest × year interaction for mycorrhizal infection (Table 1) shows, however, that the trends in infection were different between the two years, i.e. that the mean level of mycorrhizal infection was different in this set of plant species in 1988 and 1989 at one or more harvests during that year.

Table 1. *Three-way analysis of variance of the data on mycorrhizal infection of six species over two years at Wheldrake Ings*

	d.f.	*F* ratio	Sig.
Species (Sp)	5	48·23	***
Harvest (Harv)	6	2·98	**
Year (Y)	1	14·00	***
Sp × Harv	30	1·54	*
Sp × Y	5	0·82	n.s.
Harv × Y	6	8·93	***
Sp × Harv × Y	30	2·22	***

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

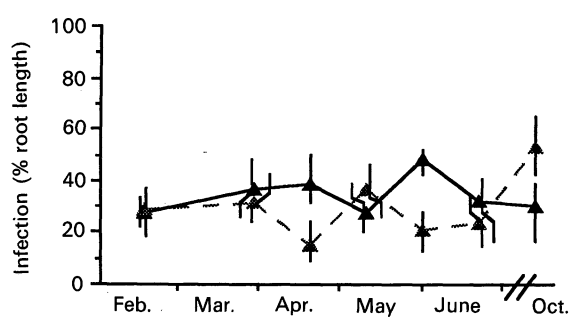

Figure 1. Mean mycorrhizal infection in six plant species at Wheldrake Ings during 1988 (---) and 1989 (——). Bars indicate 95% confidence intervals.

The hypothesis that plant species exhibit different patterns of VAM infection is supported by the significant species × harvest interaction (Table 1): interspecific differences in the seasonal pattern of mycorrhizal infection did occur. For example, *T. pratense* attained its highest level of infection during April 1988 when infection in *H. lanatus* and *F. rubra* was lower than at other times in that year (Fig. 2). In addition, *P. lanceolata* exhibited significantly greater VAM infection than *R. acetosa* during April 1988 and from the end of May to October in 1988 (Fig. 3).

The six plant species exhibited very different trends in mycorrhizal infection. *T. pratense* and *L. pratensis* showed little variation in infection either within or between years (Table 2; Fig. 2). *F. rubra* also exhibited no variation in infection either within or between years and although the harvest × year interaction is significant in this species ($P < 0{\cdot}05$) there was only one sample at the June 1989 harvest. VAM infection in *H. lanatus* showed variation both within-, and between-years, but the harvest × year interaction was not significant, indicating that although mean infection differed in the two years, the trends were consistent between years and were genuinely seasonal (Fig. 2). In contrast, *P. lanceolata* and *R. acetosa* exhibited significant harvest effects (and a year effect in the case of *P. lanceolata*) indicating within-year variation. However, the strongly significant harvest × year interactions for these two species showed that these trends were not consistent seasonal patterns (Table 2). These two species exhibited lower infection in April and late May and higher infection during October in 1989 in contrast to 1988 (Fig. 3).

Within-year variation in VAM infection

Mean VAM infection in the six species varied significantly during each year (Fig. 1). However, this variation could not be explained by a low-order polynomial in either year, i.e. no easily defined seasonal trends were observable. Although the data fitted the linear and quadratic terms in 1989 there was a significant deviation from both. The quadratic term was significant in both years.

F. rubra and *L. pratensis* exhibited no significant variation in infection during either year (Fig. 2), although significant quadratic and cubic terms could be fitted to *L. pratensis* in 1989 (Table 3). Variation

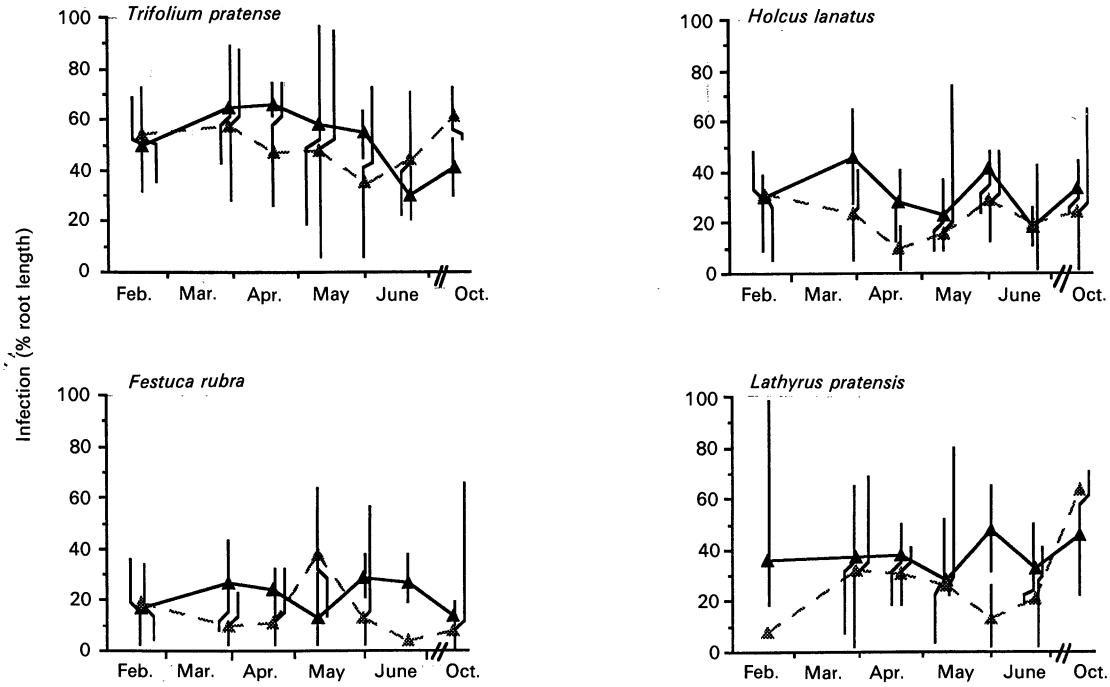

Figure 2. Mean mycorrhizal infection in *Holcus lanatus*, *Trifolium pratense*, *Festuca rubra* and *Lathyrus pratensis* during 1988 (——) and 1989 (– – –). Bars indicate 95 % confidence intervals.

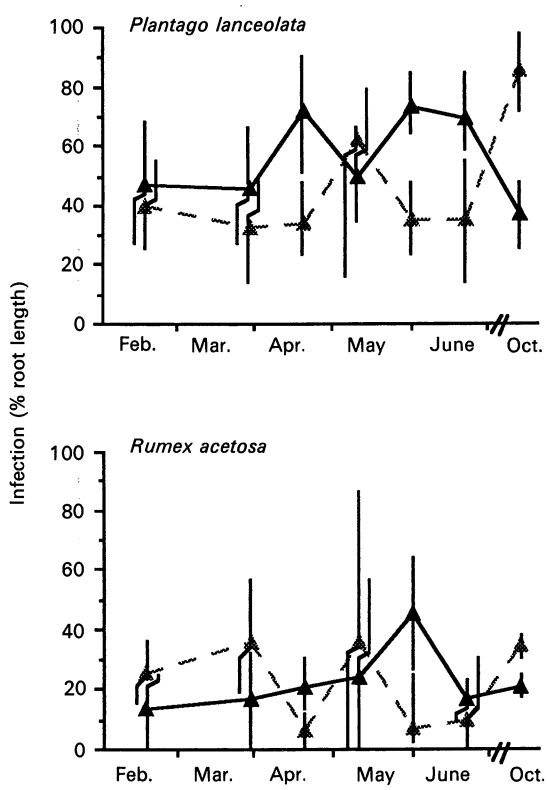

Figure 3. Mean mycorrhizal infection in *Plantago lanceolata* and *Rumex acetosa* during 1988 (– – –) and 1989 (——). Bars indicate 95 % confidence intervals.

in infection of *T. pratense* in 1988 was fitted by a cubic term (from which there was no deviation) and in *H. lanatus* by the quintic term (from which there was no deviation). Although VAM infection for these two species in 1989 showed no significant variation in an analysis of variance, the form of the curves in the two years is very similar (Fig. 2), and a quintic term could again be fitted to the data for *H. lanatus*.

Table 2. *Two-way analysis of variance of the data on mycorrhizal infection for six plant species at Wheldrake Ings*

	Harvest	Year	Harvest × year
d.f.	6	1	6
Plantago lanceolata	2·39*	6·08*	9·80***
Rumex acetosa	2·29*	0·03	6·61***
Trifolium pratense	1·99	0·37	1·75
Holcus lanatus	2·80*	6·80*	1·14
Festuca rubra	0·37	1·99	2·30*
Lathyrus pratensis	1·39	3·17	1·32

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

P. lanceolata and *R. acetosa* exhibited highly significant variation in VAM infection within each year and both species had significant peaks and troughs in infection (Fig. 3). The trends of VAM infection in *P. lanceolata* in 1988 and 1989 were significantly fitted by the second order polynomial, although in 1989 there was a large linear component to the variation as well. VAM infection in *R. acetosa* was fitted by the quadratic term in 1988 and the linear, quadratic and quintic terms in 1989 (with significant unexplained variation), indicating an extremely complex pattern.

The morphology of VAM infection

The two-way ANOVA was carried out using the morphological subdivisions of the VAM infection data in order to test the hypothesis that different

Table 3. *F ratios from one-way ANOVA of mycorrhizal infection in 1988 and 1989, using harvest date as factor*

	All spp.	Pl†	Ru	Tr	Ho	Fe	La
1988 *F* ratio (term unpartitioned)	3·19**	4·23**	2·60*	2·68*	3·00*	1·88	0·75
Linear	0·00	1·20	3·15	4·43	0·00	0·05	—
Quadratic	5·01*	13·50***	4·60*	0·17	0·58	2·92	—
Cubic	0·57	0·95	—	10·20**	3·54	0·00	—
Quartic	0·00	0·03	—	—	0·58	2·90	—
Quintic	4·29*	0·73	—	—	11·70**	0·52	—
Deviation from quintic	9·29**	8·99**	—	—	—	4·92*	—
1989 *F* ratio (term unpartitioned)	7·64***	7·99**	10·16***	1·06	1·56	1·01	2·39
Linear	17·80***	30·80***	4·49*	—	0·06	—	3·14
Quadratic	10·20***	12·60***	25·00***	—	2·47	—	4·86*
Cubic	0·07	—	0·52	—	1·87	—	4·91*
Quartic	0·47	—	3·78	—	0·01	—	—
Quintic	2·86	—	7·02*	—	4·91*	—	—
Deviation from quintic	8·45**	—	20·20***	—	—	—	—

The first five orthogonal polynomial terms have been fitted and *F* ratios are given for all terms up to the point at which no further terms or deviations from the terms are significant; these are indicated by '—'. * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† *Plantago lanceolata* (Pl), *Rumex acetosa* (Ru), *Trifolium pratense* (Tr), *Holcus lanatus* (Ho), *Festuca rubra* (Fe) and *Lathyrus pratensis* (La).

Table 4. *F values from two-way analyses of variance on morphological characteristics of total VAM infection, during 1989*

	Harvest	Species	Harvest × species
d.f.	5	5	25
Hyphae	3·58**	14·27***	1·38
Arbuscules	1·36	7·29***	0·91
Vesicles	0·46	10·53***	0·95
Vesicle width	1·05	3·55**	1·26
Vesicle length	1·17	2·76	1·58
Hyphal diameter	1·38	1·71	1·20

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

plant species have different levels of hyphae, arbuscules, and vesicles at different times of the year (Table 4). There were some highly significant interspecific differences in the root length occupied by hyphae, arbuscules and vesicles, although the length of root occupied by hyphae was the only feature of VAM morphology which exhibited variation between harvest dates (Table 4, Fig. 4). However, the harvest × species term for hyphal infection was not significant, indicating that although there was a temporal change in the amount of root occupied by VAM hyphae this trend did not vary between species. Interspecific differences also occurred in vesicle width, but vesicle length and hyphal diameter did not vary significantly between species.

DISCUSSION

The measurements of mycorrhizal infection indicated that in some species the extent of fungal infection varied greatly both within one year and in successive years, but the causes of this variation are unknown. Studies of temporal patterns of infection underline how little is known of the ecology and functioning of VAM. Overall levels of VAM infection in the major plant species of a community is a crude method of studying this symbiosis. In an attempt to understand VAM ecology and functioning in the field, a knowledge of the dynamics of mycorrhizal infection within the whole community is important. Infection may represent a significant carbon sink at the community level. Further, mycorrhizal fungi form hyphal connections between plants of the same and of different species (Newman, 1988) and nutrients can be transferred from one plant to another along these connections. If these connections are abundant in the field then this could have important implications for mycorrhizal functioning and plant performance. Thus, when considering both plant nutritional benefits of VAM and the cost to the plant in terms of photosynthate, overall levels of mycorrhizal fungi in the roots of a group of co-existing species may be as important as individual levels of infection within plant species.

Rumex acetosa has previously been reported as being non-mycorrhizal (Harley & Harley, 1987).

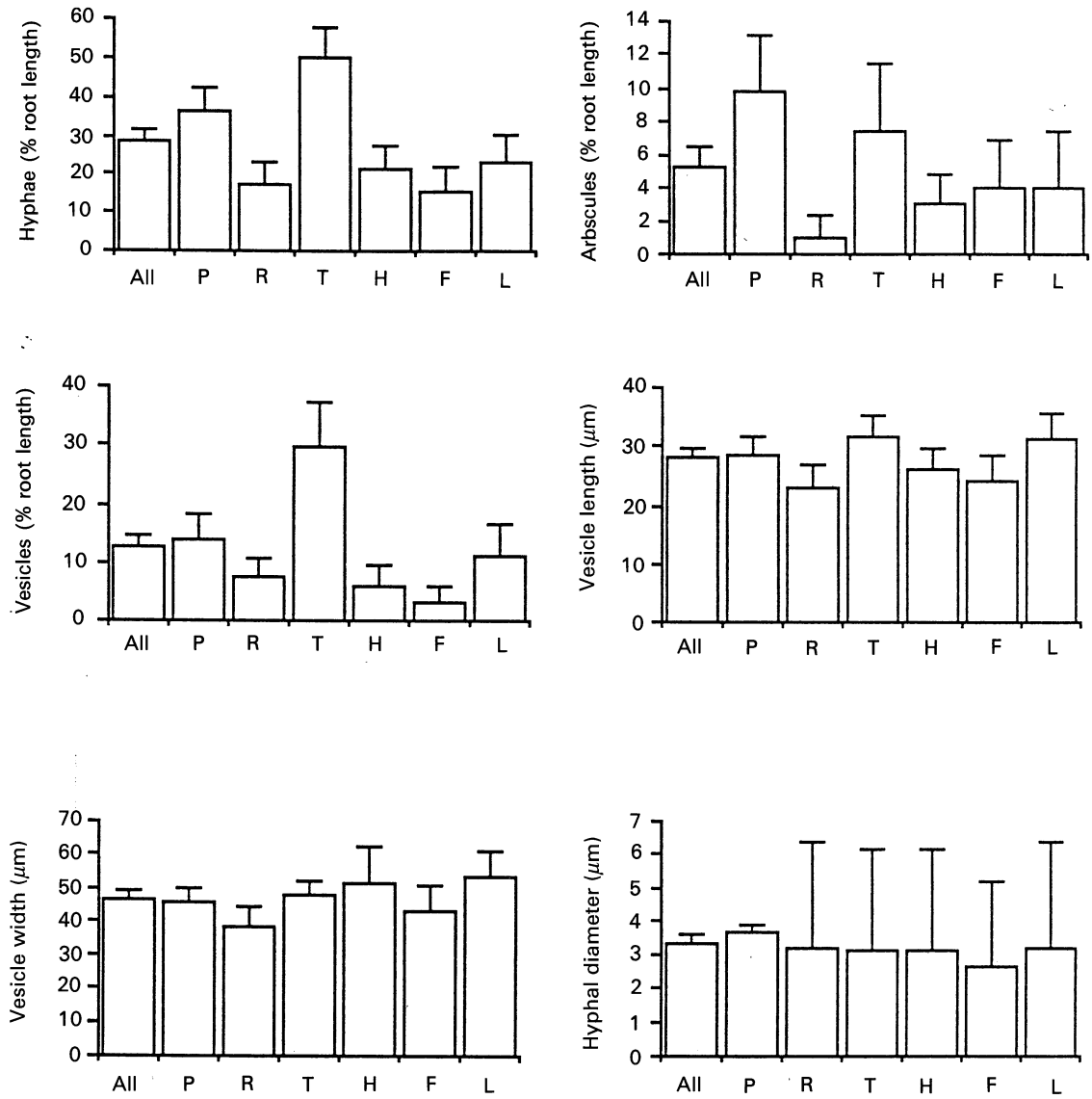

Figure 4. Mean values for the morphological characteristics of VAM infection for all species grouped together (all) and for each of the six plant species individually. Error bars show 95 % confidence intervals. *Plantago lanceolata* (P), *Rumex acetosa* (R), *Trifolium pratense* (T), *Holcus lanatus* (H), *Festuca rubra* (F) and *Lathyrus pratensis* (L).

Although the analysis on the variation of VA mycorrhizal infection in this species was carried out using the percentage root length occupied by all VA fungal structures, arbuscules were rarely observed (Fig. 4), indicating that *R. acetosa* may not form a mutualistic association.

Although the overall infection in the six plant species was different in 1988 and 1989 interspecific differences emerged when the species were considered individually. *P. lanceolata* and *R. acetosa* had highly fluctuating levels of infection within a year and although these fluctuations occurred in both years they did not follow the same trend. *P. lanceolata* showed significant temporal variation in infection in both years and appears to display a real seasonal pattern, since infection in both 1988 and 1989 was fitted by the quadratic term (Table 3). The difference in infection between the two years was large at certain times of the year (Fig. 3), but much less if the data for 1989 (an extremely early spring) are brought forward by one month (Fig. 5). *F. rubra* and *L. pratensis* exhibited little temporal variation

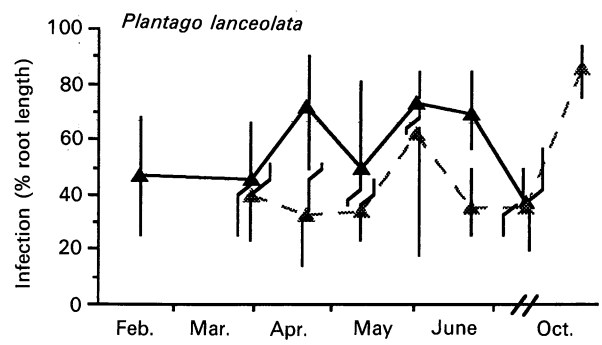

Figure 5. Mean mycorrhizal infection in *Plantago lanceolata* during 1988 (——) and with the values during 1989 (– – –) brought forward by one harvest. Bars indicate 95 % confidence intervals.

within or between years, but both *T. pratense* and *H. lanatus* exhibited variation in VAM infection in 1988 which did not recur in 1989. However, the trends in 1989 did not differ significantly from those in 1988, as shown by the lack of harvest × year interactions (Table 2). These two species (and especially *H. lanatus*) appear therefore to show genuine patterns.

Other studies have recorded peaks and troughs in VAM infection in natural ecosystems. McGonigle & Fitter (1990) examined temporal patterns of VAM infection in *H. lanatus* at the same field site between the end of April and mid-June 1986. In their study the levels of VAM infection show a definite peak in infection in May followed by a decrease in June. This is the same as observed in both years of this investigation and emphasizes the value of long-term studies of mycorrhizal occurrence in the field.

The lack of seasonality in the morphology of VAM infection, with the exception of the root length occupied by VAM hyphae, prevents the prediction of periods when the fungus is likely to influence host plant nutrition. However, a significant increase or decrease in the amount of root occupied by VAM hyphae could indicate periods when the fungus is acting to a greater or lesser extent as a carbon sink. Interspecific differences in the root length occupied by hyphae, arbuscules or vesicles and in vesicle width indicate that aspects of the fungus/plant relationship are different in different plant species.

Variation in VAM infection between plant species and seasonal changes in infection under field conditions have been ascribed to a variety of causes (Fitter, 1990). The interpretation of patterns of infection is complicated by the difficulty of distinguishing different VAM fungal species and lack of knowledge of their ecology. In this survey total mycorrhizal infection within each plant root system was measured. However, it is likely that several VAM fungal species were involved. Patterns of VAM infection could therefore be more complex than those presented here. Isolation of spores from the field site has shown that the indigenous VAM community contains a number of different species (Sanders, 1991). These spores were similar to descriptions of *Acaulospora laevis* Gerdemann & Trappe, *Glomus constrictum* Trappe, *Glomus etunicatum* Becker & Gerdemann, *Glomus invermaium* Hall, *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe, *Sclerocystis rubiformis* Gerdemann & Trappe and *Scutellospora calospora* Walker & Sanders. These fungi may infect plant species or even parts of the root system differentially.

Spatial separation of different fungal types within individual plant root systems could lead to multiple infection. The spatial distribution of spores of different mycorrhizal forming species within the soil is extremely variable (Walker, Mize & McNabb, 1982), and if spores are a common form of VAM propagation, new roots exploiting new areas of soil would be exposed to a different relative abundance of VAM species in different areas. As a consequence, the potential for different parts of a root system to become infected by different species exists. Once such patterns were established they could be maintained by re-infection from roots.

Two studies (Rosendahl, Rosendahl & Sochting 1990; McGonigle & Fitter, 1990) suggest that different VA fungal species co-exist within a root system of one plant species. In a Danish grassland community, Rosendahl *et al.* (1990) observed great differences in the level of morphologically distinct VAM infections in co-existing plants, although certain identification of the fungal species was not made. Temporal differences occurred not only in the level of distinct infections within one plant species but also appeared to be markedly different between plant species. In McGonigle & Fitter's (1990) study, also at Wheldrake Ings, infection by coarse endophytes predominated in *Phleum pratense*, *Ranunculus acris* and *P. lanceolata* whilst the majority of infection in *H. lanatus* was caused by fine endophytes; in all cases, however, both morphological types were present.

In this investigation the differences between plant species in the morphological features of VAM fungal infection (Table 4) could indicate that different plant species are infected by different mycorrhizal species or at least show some selectivity. However, these differences might also represent varied phenotypic responses of one or several fungi to different host species. Previous studies have shown that the appearance of one fungal species can be distinctly different in two host plant species (Gerdemann, 1965) and that this difference may be the result of variation in root tissue structure, producing distinct constraints on the fungus (Brundrett & Kendrick, 1990*b*). Thus, studies which primarily use morphology of infection as a basis for identification of different VAM-forming fungal species are difficult to interpret. Unless the phenotypic responses of the different fungal species within the potential range of hosts are known, recognition of distinct fungal types within the roots of different hosts cannot be achieved with certainty.

Without knowing more about the specificity and selectivity of VAM species and their hosts, the seasonal patterns of VAM infection cannot easily be interpreted. The patterns observed in this study could have been caused by selective infection by different fungal types and phenological differences between the fungi or by a simple seasonal response of a single fungal species. Equally, uniform levels of infection could mask temporally separated peaks of infection by more than one fungal type. The occurrence of either of these situations could have important consequences on the functioning of VAM within plant communities. However, it is clear that

before a good understanding of VAM function is attained more knowledge is required on the ecology of VAM fungal species.

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