New Phytol. (1993), **123**, 297–306

The development of endomycorrhizal root systems

VIII. Effects of soil phosphorus and fungal colonization on the concentration of soluble carbohydrates in roots

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(*Received 25 October 1991; accepted 6 October 1992*)

SUMMARY

Concentrations of phosphorus in shoot and soluble carbohydrates (fructose, glucose, sucrose and fructans) in root were measured in non-mycorrhizal and vesicular–arbuscular (VA) mycorrhizal (*Glomus mosseae*) leek plants (*Allium porrum*) raised at six concentrations of soil phosphate. In conditions when an increased concentration of soil phosphate reduced VA mycorrhizal infection, the concentrations of soluble carbohydrates in the root were at a maximum. Therefore the hypothesis that greater concentrations of soluble carbohydrates in roots favour VA mycorrhizal infection is discounted. There was a specific effect of VA mycorrhizas, in that infected roots contained a larger concentration of sucrose than did uninfected roots, in plants with similar phosphorus concentrations in dry matter of shoots.

We conclude, first, that increased phosphorus supply from either phosphate addition to soil or VA mycorrhizal infection increases concentration of soluble carbohydrates in leek roots and, secondly, that the VA mycorrhizal root behaves as a particularly strong physiological sink when there is an excess concentration of sucrose in the host.

Key words: Soil phosphorus, vesicular–arbuscular mycorrhiza, soluble carbohydrate, *Allium porrum*, *Glomus mosseae*.

INTRODUCTION

There are three major effects of large soil P concentration on the colonization of roots by vesicular–arbuscular (VA) mycorrhizal fungi: an increased delay in establishing infection, a decrease in the rate of extension of infection along the root cortex, and a reduction in the intensity of internal infection (Amijee, Tinker & Stribley, 1989). These observations suggest a nutritional effect, for studies of other filamentous fungi growing on solid media show that reduced branching and linear rates of colony extension characteristically occur when substrates are in short supply (Bull & Trinci, 1977; Prosser, 1983).

The supply of carbohydrates from host to mycorrhizal fungus has frequently been suggested as a mechanism by which the host may control spread of the fungus (Bjorkman, 1942). Later, Schrader (1958) and Peuss (1958) used it to account for effects of light intensity and defoliation respectively. Two relevant theories have been postulated. Robson and his colleagues (Jasper, Robson & Abbott, 1979; Same, Robson & Abbott, 1983; Thomson, Robson & Abbott, 1986) observed that plants grown on small soil P concentration contained the largest concentrations of soluble carbohydrate, and the largest percentage of mycorrhizal colonization. They postulated that this relationship was casual. However, the percentage colonization is a complex measure to use, as it depends upon both fungal and root growth rates (Buwalda, Stribley & Tinker, 1984; Amijee, Stribley & Tinker, 1986) and therefore the conclusion that fungal growth rate was improved is not

Table 1. *Phosphorus application (as $Ca(H_2PO_4)_2.H_2O$), shoot dry weight of non-mycorrhizal (NM) and mycorrhizal (M) plants at 52 d and VA mycorrhizal colonization at 32 d and 52 d of leeks* (Allium porrum) *at six soil P treatments*

P applied (mg kg^{-1})	Shoot dry wt (g)*		VA mycorrhizal colonization* (%)†		(mm d^{-1} $front^{-1}$)‡	
	NM	M	32 d	52 d	32 d	52 d
0 [P0]	0·16 (0·030)	0·39 (0·023)	23·1 (2·20)	16·5 (0·96)	0·9 (0·06)	0·9 (0·12)
150 [P1]	0·35 (0·042)	0·47 (0·048)	15·5 (2·36)	21·8 (2·12)	1·0 (0·04)	1·0 (0·06)
300 [P2]	0·54 (0·049)	0·61 (0·021)	13·3 (0·73)	17·7 (2·29)	0·8 (0·02)	1·0 (0·06)
450 [P3]	0·88 (0·092)	0·76 (0·116)	5·3 (0·85)	7·7 (1·83)	0·3 (0·01)	0·4 (0·03)
600 [P4]	0·68 (0·068)	0·36 (0·016)	1·5 (0·87)	5·7 (0·88)	0·4 (0·03)	0·4 (0·03)
750 [P5]	0·26 (0·031)	0·29 (0·059)	0·2 (0·10)	3·8 (0·77)	0·3 (0·02)	0·5 (0·15)

SE in parentheses ($n = 4$).
* Data from Amijee, Tinker & Stribley (1989).
† Percentage of total root with internal hyphae.
‡ Mean rate of extension per infection front on main axes of roots.

warranted. Further, the methods of determining the concentration of carbohydrates from crude extracts were inadequate in comparison with detection techniques currently available. We regard the mean extension rate of the fungus along the root cortex as the most direct measure of this relative rate of increase of the fungus.

A related theory put forward by Menge and his co-authors (Ratnayake, Leonard & Menge, 1978; Graham, Leonard & Menge, 1981, 1982; Ferguson & Menge, 1982; Johnson *et al.*, 1982) postulated that the exudation of carbohydrates from roots is the important variable, as they found correlations between the rate of exudation and the percentage of mycorrhizal colonization. Similar reservations as above apply to the use of percentage of mycorrhizal colonization and to analytical methods used to assess carbohydrate requirement by the fungus. Recently, Schwab, Menge & Tinker (1991) reviewed and expressed some doubts on these theories. Tester *et al.* (1986) aptly pointed out that 'correlations between exudation, carbohydrate concentration and mycorrhizal infection require re-examination using methods which measure unequivocally the concentrations of well defined classes of compounds'.

This paper presents measurements made on the plant material from our previous experiment (Amijee *et al.*, 1989) to study the relationship between concentration of P in shoots, concentration of fructose, glucose, sucrose and fructans in roots and VA mycorrhizal colonization of roots at different soil P concentrations.

MATERIALS AND METHODS

General

Non-mycorrhizal (NM) and mycorrhizal (M) plant material of leek (*Allium porrum* L., cv. Musselburgh) raised at six soil P concentrations was collected from a larger experiment to study the effects of P on VA mycorrhizal colonization (Amijee *et al.*, 1989). Plants, at one seedling per pot, were grown in cylindrical PVC pots containing a 2:1 (w/w) mixture of gamma-irradiated (1 Mrad) soil (Cottenham Series, Catt *et al.*, 1980) and steam-sterilized sand supplemented with 0 (P0), 150 (P1), 300 (P2), 450 (P3), 600 (P4) and 750 (P5) mg P kg^{-1} soil (as monocalcium diphosphate ($Ca(H_2PO_4)_2.H_2O$)). The mycorrhizal treatment was inoculated 3 cm below the soil surface with a dense layer of inoculum of *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann and Trappe.

The pots were placed in a controlled environment cabinet at 20/16 °C with a 14 h photoperiod and watered to weight daily. Because of practical limitations, two replicates of NM and M plants harvested at 32 d and 52 d were used for the measurements of P concentration in dry shoots and concentration of ethanol-soluble carbohydrates in fresh roots. Hence the data are presented as individual values obtained from each replicate rather than as treatment means. These measurements complement data on shoot growth and root colonization (as percentage colonization of total root and mean rate of extension per infection front on main axes of roots, Amijee, 1986) collected from the other four replicate plants of the M treatment raised at the six soil P concentrations. Full details of these measurements, experimental design and conditions for plant growth are reported in Amijee *et al.* (1989).

Phosphorus in shoots

Phosphorus was extracted from oven-dried and ground shoot material (80 °C for 24 h). Ten ml of nitric/perchloric acid mixture (13 vol of perchloric acid, primar 70 %, to 87 vol of nitric acid, Aristar s.g. 1·41) were poured into a 20 ml graduated boiling

tube containing 0·100 g of plant material. The tubes were initially heated to 60 °C for 3 h, followed by a rise in temperature to 100 °C, 120 °C and 240 °C over a period of 1, 2 and 5 h respectively, until near-dryness was reached. This digestion was carried out in a fume hood fitted with a back-wash. The tubes were allowed to cool before adding 5 ml of HCl (20 %, v/v), mixed and reheated at 80 °C for 30 min. Distilled water was added to make up the extract to 20 ml before determining the total P concentration in shoot dry matter on the ICP atomic emission spectrometer (method of S. P. McGrath, personal communication). Concentrations of Cu and Zn were also determined on the same aliquots to test the possibility that large concentrations of soil P might induce Cu and Zn deficiency (Bingham, 1966), and that this might be affected by VA mycorrhizal infection.

Soluble carbohydrates in roots

Fresh root material, washed free of soil (Amijee *et al.*, 1989), was weighed at harvest, immediately frozen in liquid nitrogen and stored at −72 °C to stop enzyme activity. The frozen root material was placed in a boiling tube containing 5 ml of hot (70 °C) ethanol (80 %, v/v) for 30 min to extract ethanol-soluble carbohydrates. After cooling, the extract was transferred to small glass vials. Ethanol was removed by evaporation under an air-stream and the residue was lyophilized before silylating by addition of 50 μl of pyridine and 50 μl of trimethyl-silylimidazole (TMSI). The samples were mixed, heated at 80 °C for 15 min and allowed to stand overnight at room temperature (method of L. Skot, personal communication). One μl of the sample was injected into a gas chromatograph fitted with a flame ionization detector (Perkin-Elmer Sigma 3B, USA) and connected to a chart recorder. A stainless steel column (length = 2 m, internal diameter = 2 mm) containing 3 % OV 17 on chromosorb WHP of 100–120 mesh (Phase Separations, UK) was used to separate the carbohydrates. The column temperature was initially 145 °C for 2 min, then rising to 250 °C at a rate of 7·5 °C min^{-1} and remaining at 250 °C for 4 min. Oxygen-free nitrogen was used as a carrier gas at a rate of 65 ml min^{-1}.

The TMSI derivatives of each carbohydrate in the ethanol extract of roots were identified by paper chromatography, co-chromatography and comparisons of retention time with known standards (Amijee, 1986). Phenyl β-D-glucoside was used as an internal standard. Amounts of fructose, glucose and sucrose were measured by calculating the ratio between peak height of each carbohydrate to that of the internal standard. Where several peaks were produced by a single carbohydrate (e.g. fructose and glucose), a summation of all peak height ratios was used. Standard curves were prepared for each carbohydrate and the extracted amount quantified according to the unit weight of fresh root (Ericsson, Hansen & Dalgaard, 1978).

The amount of free and combined fructose (fructan) was estimated in the ethanol (80 %, v/v) extracts from a single replicate of NM and M leek roots harvested at 52 d. Because of the limited amount of root material, it was not possible to use a less polar solvent, as well as ethanol, to extract high-molecular-weight fructans. The samples were separated by gel filtration on a column of Bio-Gel P2 (Pollock, 1979, 1982), before estimating free and combined fructose by the ketose-specific modification of the anthrone method (Jermyn, 1956).

RESULTS

At 32 d, added P caused a gradual decrease in percentage of root colonized by *Glomus mosseae*, but at 52 d, percentage of root colonized was increased with P applications up to P2, followed by a decline at larger rates of P application (Table 1). The mean rate of extension per infection front on the main axes of

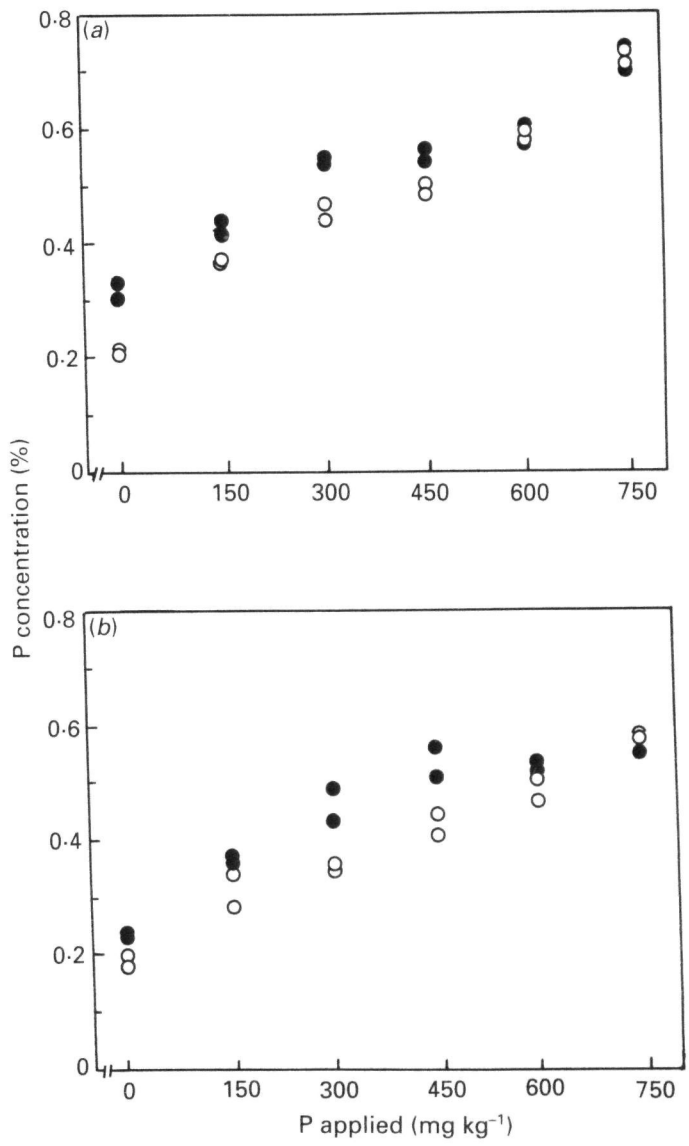

Figure 1. Phosphorus concentration at (*a*) 32 d and (*b*) 52 d in shoots (dry-matter basis) of non-mycorrhizal (○) and mycorrhizal (●) leeks (*Allium porrum*) at six rates of applied P.

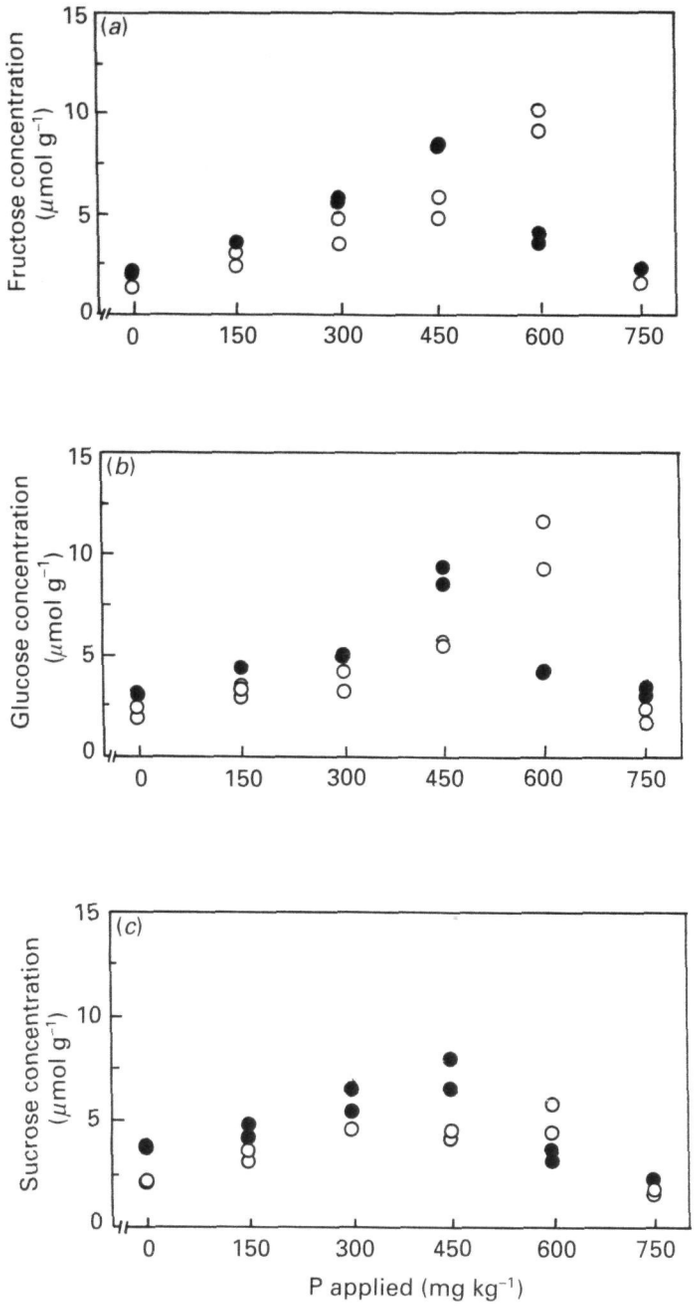

Figure 2. Concentrations of (*a*) fructose, (*b*) glucose and (*c*) sucrose at 32 d in roots (fresh-weight basis) of non-mycorrhizal (○) and mycorrhizal (●) leeks (*Allium porrum*) at six rates of applied P.

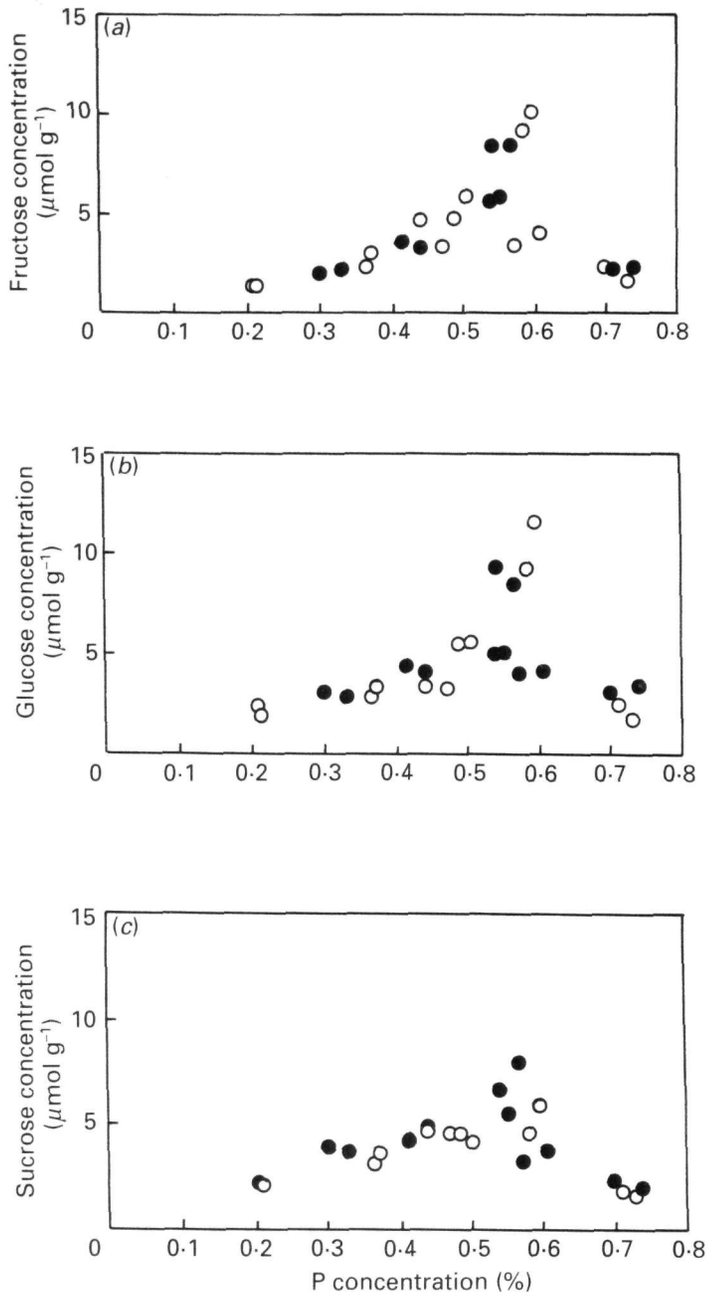

Figure 3. Concentrations of (*a*) fructose, (*b*) glucose and (*c*) sucrose at 32 d in roots (fresh-weight basis) of non-mycorrhizal (○) and mycorrhizal (●) leeks (*Allium porrum*) as a function of P concentration in shoots (dry-matter basis).

roots remained at approx. 1 mm d^{-1} per front at P0, P1 and P2, but it was reduced to approximately 0·4 mm d^{-1} per front at P3, P4 and P5 (Table 1).

In both NM and M plants, concentration of P in dry matter of shoots increased with increasing concentrations of soil P (Fig. 1). Mycorrhizal infection also increased P concentration of shoots, although this effect decreased with increasing concentrations of soil P (Fig. 1). There was a decline in shoot P concentration between 32 d and 52 d at all soil P concentrations. Concentrations of Cu and Zn did not differ between NM and M plants, or between soil P concentrations (Amijee, 1986).

Paper chromatography, co-chromatography and comparisons of retention times by gas chromatography gave good separation of the soluble carbohydrates and verified that the three detectable carbohydrates in the ethanol extracts of NM and M roots (fresh weight basis) were fructose, glucose and sucrose (Amijee, 1986). No polyols or soluble carbohydrates specific to the VA mycorrhizal fungus were detected in mycorrhizal roots. The concentrations of fructose, glucose and sucrose increased with P application initially (Figs 2 and 4), before declining at P4 and P5. Mycorrhizal roots had greater concentrations of fructose, glucose and sucrose than did NM roots raised at similar soil P concentrations (Figs 2 and 4). However, concentrations of fructose and glucose were no greater in M roots when the data were expressed as a function of P concentration in shoots (Figs 3 and 5). There was an exception with sucrose, for its concentration continued to remain large in M roots when compared with NM plants of similar shoot P concentration particularly at 52 d (Fig. 5*c*).

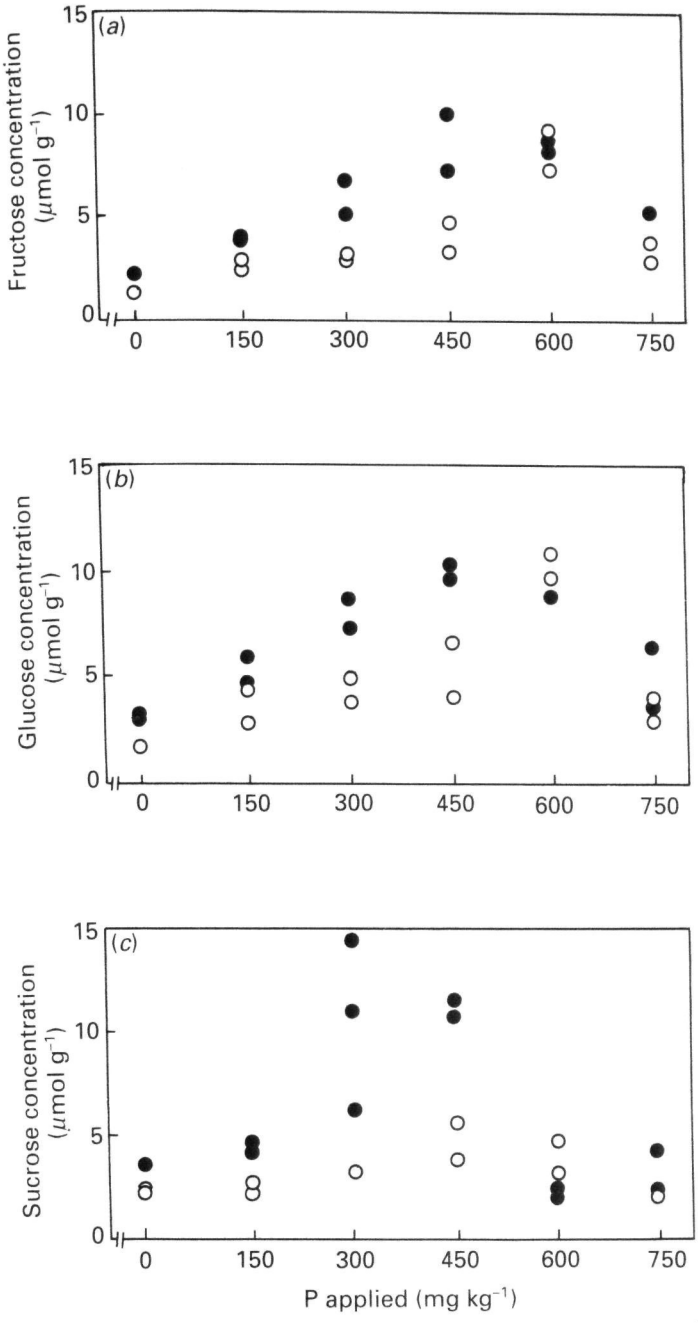

Figure 4. Concentrations of (*a*) fructose, (*b*) glucose and (*c*) sucrose at 52 d in roots (fresh-weight basis) of non-mycorrhizal (○) and mycorrhizal (●) leeks (*Allium porrum*) at six rates of applied P.

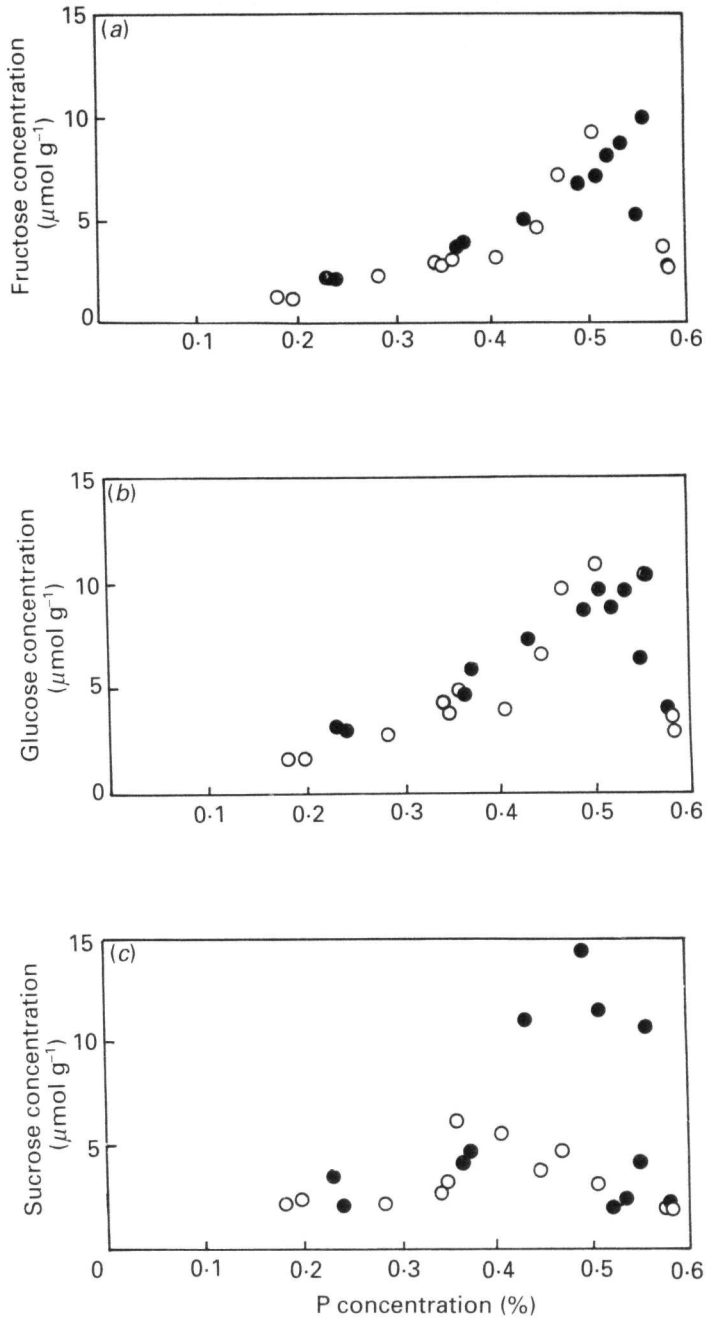

Figure 5. Concentrations of (*a*) fructose, (*b*) glucose and (*c*) sucrose at 52 d in roots (fresh-weight basis) of non-mycorrhizal (○) and mycorrhizal (●) leeks (*Allium porrum*) as a function of P concentration in shoots (dry-matter basis).

Fructans were present in the ethanol extracts of NM and M roots and were in the range of 3 to about 10 degrees of polymerization (Amijee, 1986). The concentration of fructans also increased with P application initially before declining at very large concentrations of soil P (Fig. 6*a*). There was a difference in concentration of fructans between NM and M roots at similar soil P concentration, but this difference was eliminated when NM and M roots of similar shoot P concentration were compared (Fig. 6*b*).

To establish whether VA mycorrhizal fungus was affected by soluble carbohydrate concentrations in the root, measurements of the extension rate of infection fronts on the main axes of roots were compared with the concentration of sucrose in the roots (Fig. 7). The poor correlation ($r^2 = 0{\cdot}005$ at 32 d, $r^2 = 0{\cdot}025$ at 52 d) between these two variables suggests that sucrose concentration in the root was not an important rate-limiting factor in VA mycorrhizal colonization.

DISCUSSION

Effects of phosphorus and VA mycorrhizas on plant growth

Phosphate application increased growth of leek plants to a maximum at P3 (soil bicarbonate-soluble P = 208 mg kg^{-1}), but at large soil P concentrations P became toxic and reduced plant growth (Amijee *et al.*, 1989). Colonization of leek roots by *Glomus mosseae* was reduced by P application, with a marked decrease when bicarbonate-soluble P exceeded 140 mg kg^{-1} (> P2, Table 1; Amijee *et al.*, 1989). It

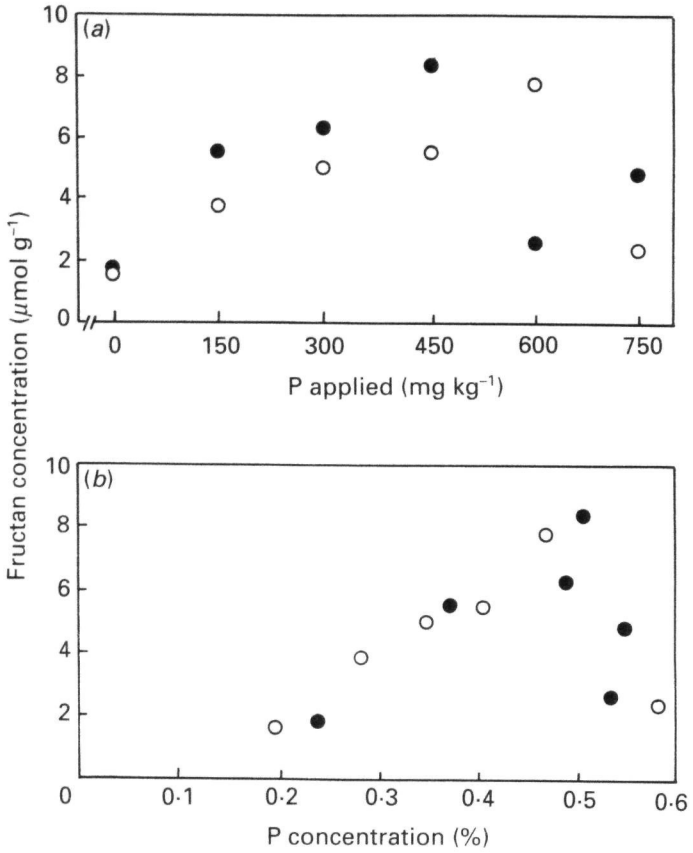

Figure 6. Concentrations of fructans at 52 d in roots (fresh-weight basis) of non-mycorrhizal (○) and mycorrhizal (●) leeks (*Allium porrum*) at (*a*) six rates of applied P and (*b*) as a function of P concentration in shoots (dry-matter basis).

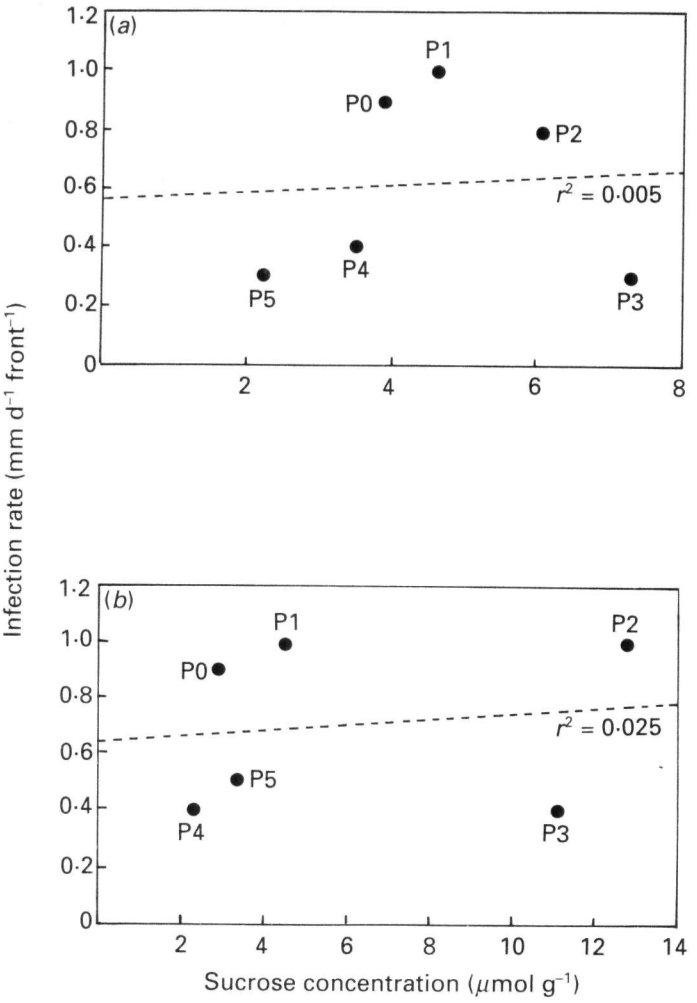

Figure 7. Rates of mycorrhizal infection on the main axes at (*a*) 32 d and (*b*) 52 d as a function of sucrose concentration in roots of leeks (*Allium porrum*) at six rates of applied P.

was accompanied by an increase in the delay of establishment of infection, a decrease in the intensity of internal colonization and a particularly sharp decrease in the rate of lateral extension of infection (Table 1). These effects of P upon VA mycorrhizal colonization are discussed in detail by Amijee *et al.* (1989).

The concentration of P in shoots of NM and M plants (dry weight basis) increased with increasing concentrations of soil P (Fig. 1). There was an additional effect in M plants, where mycorrhizal infection enhanced P uptake at small concentration in soil P (Fig. 1). This effect disappeared at large concentration of soil P when development of mycorrhizal infection was reduced (Fig. 1; Table 1). The temporary increase in shoot P concentration at 32 d following establishment of infection in M plants has been observed before (Tinker, Stribley & Snellgrove, 1982; Stribley & Snellgrove, 1985, 1986), and is generally believed to be a result of a delay in the operation of the regulatory mechanism of P uptake by mycorrhizal infection.

The ethanol-soluble carbohydrates found in roots of leek, whether NM or M, were fructose, glucose, sucrose and fructan. No specific fungal carbohydrates such as trehalose (Amijee & Stribley, 1987) were detected in mycorrhizal roots. This agrees with most other studies on soluble carbohydrates of *Allium* species, in particular *Allium cepa* (Bacon, 1957, 1959; Bose & Shrivastava, 1961; Lolas & Markakis, 1973; Darbyshire & Henry, 1978). Plants of the genus *Allium* do not contain starch (Gates & Simpson, 1968; Darbyshire & Henry, 1981): the only non-structural polysaccharide is fructan (Lewis, 1984; Pollock, 1984). The function of fructans in plants is little understood. Housley & Pollock (1985) have suggested that synthesis of fructans helps to maintain a constant cytoplasmic concentration of sucrose.

The results show that concentration of soluble carbohydrates in roots of NM and M plants increased with soil P concentrations to a maximum at P3 or P4 and then declined (Figs 2, 4 and 6). Mycorrhizal infection also increased the concentration of soluble carbohydrates up to P3 soil P concentration. The decrease in the concentration of soluble carbohydrates at very large concentrations of soil P corresponded with a decrease in the mass of shoot (Table 1; Amijee *et al.*, 1989). Therefore, concentration of soluble carbohydrates changed in the same way as shoot growth.

Carbohydrate theories and VA mycorrhizal colonization

Bjorkman (1942) proposed that large concentrations of carbohydrate in the root favour ectomycorrhizal

colonization. This basic theory has been developed by others (Hacskaylo, 1985; Nylund, 1988). Lewis (1975) pointed out that Bjorkman's hypothesis embraces two separate considerations. First, there might be an initial causal relationship between carbohydrate concentration and infection, and secondly, when infection becomes established this in turn might influence the carbohydrate concentration in the infected tissue.

Our data do not support the idea that soluble carbohydrates in leek roots influence mycorrhizal infection, because concentrations were increased by addition of P in both NM and M plants, and they continued to increase when added soil P decreased mycorrhizal colonization (Table 1; Figs 2, 4 and 6). Similar studies by Siqueira, Hubbell & Valle (1984) on *Glycine max*, and by Ocampo & Azcon (1985) on *Triticum vulgare* also indicated that soluble carbohydrate concentrations in roots of NM and M plants were increased by improved P nutrition. However, these results are in contrast with those of Robson *et al.* (Jasper *et al.*, 1979; Same *et al.*, 1983; Thomson *et al.*, 1986; Thomson, Robson & Abbott, 1991), who reported that soluble carbohydrates in roots of *Trifolium subterraneum* L. decreased as the P supply increased. The results of Robson and his colleagues are surprising in view of the relationship between cytoplasmic P_i and photosynthesis (Preiss, 1984; Sivak & Walker, 1986; Walker & Sivak, 1986). At small concentrations of P_i in the cytoplasm, the phosphate translocator is unable to remove triose P from the chloroplast, hence export of fixed carbon for sucrose synthesis is reduced: and in those plants that form it, starch accumulates in the chloroplast as a result of the diversion of fixed carbon (Herold, Lewis & Walker, 1976; Herold, 1980). Hence it would be expected that, in plants deficient in phosphate, the flux of sucrose into the plant would be reduced. The mean concentration of soluble carbohydrate is however a balance between the flux into the root and the rate at which it is used in respiration and growth. Therefore different results with different species and conditions are conceivable.

The other important carbohydrate-based theory relating to the control of VA mycorrhizal infection was proposed by Menge and his colleagues (Schwab *et al.*, 1991), who suggested that the exudation of soluble carbon compounds, assumed to be fungal substrates, into the rhizosphere is reduced under high P nutrition. This was based on the properties of the cell membrane, although the concentration of soluble carbohydrates in roots would also be expected to have an effect. Their theory might explain a range of environmental effects, but can be criticized particularly with regard to P supply. Exudation from P-deficient plants might initially be large and therefore favour early stages of infection, but the resulting fungal colonization should improve P nutrition of the host, thereby reducing further exudation and later stages of VA mycorrhizal colonization. It is further weakened by the evidence that plants grown under low Ca supply, where leaky membranes might be expected, were highly resistant to infection (Hepper & O'Shea, 1984).

It should be emphasized that the use of percentage colonization of a root system is inherently unsatisfactory as a measure of fungal growth rate, because it is a complex result of the growth rate of the host and the endophyte, and it depends upon the point which the system has reached in the typical sigmoidal curve relating fungal colonization with time (Huisman, 1982). We therefore regard the mean extension rate of the infection fronts (Table 1) as a less arbitrary measure of fungal growth, even though both measures in this experiment apparently agree in showing a sharp decrease between P2 and P3 (Table 1). However, most importantly, our results clearly show that the extension rate of fungal infection was not simply related to sucrose concentration in the root (Fig. 7). This conflicts with previous carbohydrate-based theories, especially where reports have implied that fungal growth rates depend upon carbohydrate concentration in the root. It is possible that the initial increase in percentage colonization often observed with the first increment of added P to soils of small P concentrations (Amijee *et al.*, 1989) could be due to the increased carbohydrate concentrations from the initially very low levels, but this cannot be determined from our results.

VA mycorrhizal root as a physiological sink

The effect of P upon carbohydrates in roots suggests that improved P nutrition may partly account for the increase in carbohydrate as a result of mycorrhizal infection. It is further supported by the finding that when the concentrations of fructose, glucose and fructans in roots were related to the P concentration in shoots (Figs 3, 5 and 6), the data for NM and M treatments coincided. However, the effect of mycorrhizal infection on the concentration of sucrose cannot be wholly explained in terms of P nutrition, because at 52 d M plants contained a markedly larger concentration of sucrose in roots (at P2 and P3) than did NM plants with similar concentrations of P in shoots (Fig. 5*c*).

The experiments of Snellgrove *et al.* (1982), in which NM plants grew at similar rates to M plants, indicated that the respiration rate per unit weight of M root was greater than for NM root. This would suggest an increase in the local consumption of carbohydrate. An increase in the concentration of sucrose therefore implies that the flux of sucrose into the M root must have increased also. Consequently, M roots act as a stronger physiological sink because of a directional and specific mechanism which produces a larger concentration of sucrose. Such an

effect would only be observed when there was both a high level of soluble carbohydrates in the host and reasonably high levels of infection, e.g. at P2 and P3. It would not be seen when concentration of soluble carbohydrates in the host was limiting growth, e.g. at P0 and P1. At P4 and P5, no accumulation of sucrose would be expected because fungal colonization was small, and concentration of soluble carbohydrates as well as host growth was reduced, probably as a result of P toxicity.

A different approach to test whether M roots act as a sink for fixed carbon was used by Koch & Johnson (1984). They inoculated one half of a split root system of a citrus plant with *Glomus intraradices*, and found that even though both halves of the root system contained a similar P concentration, fixed ^{14}C-labelled photosynthates were preferentially translocated to the mycorrhizal half. They inferred from this that M roots were a preferential sink of carbon, although there was no consistent increase in carbohydrate concentration in roots. This interpretation is equivocal, for Drew & Saker (1978) showed that a local supply of P to a root causes it to branch profusely in that region, implying that a local high inflow of P attracts carbon to that site. The high P inflow in the mycorrhizal half of a split root system might therefore alone be sufficient to explain the diversion of fixed carbon. However, a specific effect of mycorrhizal infection on sucrose concentration (which cannot be explained by shoot P concentration alone) occurred at P2 and P3, where there was no correlation with P inflows (Amijee, 1986).

Other studies on leek and different host species (*Allium cepa*, Hayman, 1974; *Araucaria cunninghamii*, Bevege, Bowen & Skinner, 1975; *Triticum vulgare*, Azcon & Ocampo, 1981; *Citrus* species, Nemec & Guy, 1982; *Allium porrum*, Jabaji-Hare & Kendrick, 1985) have failed to demonstrate an increase in carbohydrate concentrations in roots after mycorrhizal infection. Gas chromatography was used in our studies, whereas some variation of the anthrone technique (Morris, 1948) was used to measure carbohydrate concentrations in the above studies. The anthrone method is thought to be unreliable: it can be sensitive to small variations in the heating conditions used and it can also be affected by interfering substances, as it is not specific for carbohydrates (Yemm & Willis, 1954). Attempts to separate each carbohydrate fraction before colorimetric assay were not made in the above studies, and therefore other substances might have confounded their results. Further, care was taken to ensure that enzyme activity was stopped immediately after excision of roots in our study, whereas in the experiments of Hayman (1974) and Nemec & Guy (1982), roots were dried before assay, and this might have changed the carbohydrate fraction in roots after harvest. However, it is possible that others might have different findings, because of differences in experimental conditions which did not give treatments with excess sucrose concentrations.

The diversion of host assimilates to the site of infection in mycorrhizal plants should not be surprising, for such altered patterns of translocation are found in different types of antagonistic and mutualistic infections of vascular plants by biotrophic fungi (Smith, Muscatine & Lewis, 1969; Whipps & Lewis, 1981; Manners & Gay, 1982, 1983; Farrar, 1984).

Our conclusions are as follows. (1) Increased P supply resulting from either P addition to the soil or VA mycorrhizal infection progressively increases the concentration of soluble carbohydrates in roots of leek plants up to the point where the added P reduces plant growth. (2) Neither the rate of spread of the VA mycorrhizal fungus nor the percentage of root colonized by the fungus correlates well with the concentration of soluble carbohydrates in the root. (3) The concentration of soluble carbohydrates was generally similar for NM and M plants with the same shoot P concentrations. However, concentration of sucrose in M plants at P2 and P3 was considerably greater than in NM plants. This suggests a specific directing mechanism that overcompensates for the sucrose demand by the fungal symbiont when there is ample carbohydrate being formed and reasonable levels of mycorrhizal infection. (4) The results do not support the Bjorkman hypothesis or other related theories linking mycorrhizal development directly and simply to carbohydrate supply.

ACKNOWLEDGEMENTS

F. Amijee was in receipt of an Agricultural and Food Research Council grant during the course of this work. We thank Dr C. J. Pollock for analyses of fructan concentration in roots and Dr F. E. Sanders for useful discussions.

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