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The development of extraradical hyphae in relation to the response of cowpea to VA mycorrhizal infection

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INTRODUCTION

The principal way in which VA mycorrhizal fungi benefit plant growth is by increasing the volume of soil explored. Consequently an effective VA mycorrhizal fungus should be able to form an extensive network of hyphae, called external or extraradical hyphae, in the soil in and beyond the rhizosphere. Thus it would be most useful to know the spatial distribution of VA mycorrhizal hyphae in soil or at least to have a measure of the weight or length of these hyphae. Very few authors have attempted to assess the amount of extraradical hyphae and relate it to the beneficial effect of mycorrhizal infection on the plant. Comparing four mycorrhizal fungi, SANDERS *et al.* (1977) found that three of them with similar excernal mycelium equally improved plant growth and one with little external mycelium produced no growth increase (onion). They concluded their study by stressing the vital role of the external hyphae which enhance nutrient uptake and transfer to the system. Using soybean BETHLENFALVAY *et al.* (1982) found that the progress of extraradical hyphae reflected the plant growth enhancement to a much greater extent than that of internal hyphae.

In the present communication we report observations on the time course of the intra- and extraradical development of *Glomus mosseae* infecting cowpea (*Vigna unguiculata*) and the simultaneous effect of the VA mycorrhizal infection on N_2 fixation, growth, F and water content of the host. We also attempt to reappraise the interpretation of the results obtained with the different methods of assessment of VA mycorrhizal infection.

MATERIAL AND METHODS

Cowpea, cv 58-185 from the National Centre of Agronomic Research Bambey, Senegal, was grown in pots filled with 1 kg of a sterile typical psamment soil (DECK soil). Details about the growth conditions and inoculation are reported elsewhere (GUEYE *et al.*, 1985). Two treatments were compared: inoculation with *Rhizobium* (ORS407) alone (R) and dual inoculation with *Rhizobium* and *Glomus mosseae* (RM).

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Successive harvests of five randomly selected replicates of each treatment started 5 days after planting and continued at 5-day intervals. At each harvest, nodules, shoot dry weight, P and water content of shoots and acetylene reduction activity were assessed. Root samples were removed for infection assessment after clearing and staining with trypan blue in lactophenol. Frequency (percentage of infected root pieces 0.3 mm in length) and intensity (percentage of infected root volume) were then measured according to OLLIVER et *al.* (1983). The length of extraradical hyphae per cm of root was estimated as follows. After the entire root system of each plant had been gently washed from the soil, randomly selected samples of roots (ca 50 cm in total length) were stained with trypan blue as above. All the extraradical hyphae from the stained roots were collected under a dissecting microscope and homogenized in 5ml of water at high speed. Then five 25 µl aliquots were removed and deposited onto a thin layer of 1.5% sterile water agar in a petri dish. After the liquid was completely absorbed, the agar layer bearing the mycelium from a 25 µl aliquot was microscopically observed at low magnification. Total length of the hyphae was evaluated using an eyepiece micrometer and expressed as cm of hyphae per cm of root.

RESULTS

Intraradical infection of *Glomus mosseae*. The frequency percentage was nil at day 5, but it increased rapidly at day 10 (39%) and was close to maximal at day 15 (87%); then it remained at the highest level (100%) up to the end of the experiment. The intensity percentage was nil at day 5; it increased rapidly from day 10 (6%) to day 15, remained at the same level (41 - 53%) between day 15 and 3.5, then reached a plateau (ca 75%) where it stayed up to day 50 (Table 1).

Extraradical development of *Glomus mosseae*. The pattern of extraradical hyphae length was quite different. The extraradical hyphae started to develop significantly *only* at day 20, then they rapidly reached a plateau (5 - 6 cm/cm of root) up to day 50 (Table 1).

Nodulation and acetylene reduction activity. Nodule dry weight of dually inoculated plants was only ca 6 mg per plant on day 15 but it increased sharply to 35 mg on day 20 and reached a plateau (ca 100 mg) where it stayed up to day 50. Nodule dry weight of plants inoculated with *Rhizobium* alone increased more slowly: from 5 mg on day 15 it reached only 15 mg on day 25 and then remained *more or* less constant around 50 - 60 mg (Table 1). Similarly the increase of N_2-fixing activity expressed as ARA (acetylene reduction activity) was relatively more rapid in dually inoculated plants than in non-mycorrhizal ones between day 15 and 25. Thus in mycorrhizal plants, nodulation and N_2 fixation appeared to be markedly increased from day 20, which coincided with the onset of the extension of extraradical hyphae (Fig. 1). There was no evident relation between the N_2-fixing activity or nodulation and the development of intraradical infection as measured by frequency or intensity index.

Plant grouth. Surprisingly the beneficial effect of mycorrhizal infection was significant only from day 45 whereas the effect on nodulation had occurred much earlier. The delayed response of plant growth to mycorrhizal infection could be attributed to the large requirements of the nodules for P and perhaps to the C drain by *Glomus mosseae* and ***Rhizobium.***

Table 1. Shoot weight, nodule weight and mycorrhizal infection of *Vigna unguiculata* cv. 58-185 inoculated with *Glomus mosseae* and cultivated in sterile Bambey Deck soil

Table 1. Shoot weiit, nodule weight and mycorrhizal infection of Vigna unguiculata cv. 58-185 inoculate with Glomus mosseae and cultivated in sterile Bambey Deck soil

Days	Treaments	Shoot dry weight (g per plant)	Nodules dry weight (mg per plant)	Extraradical infection (cm of hyphae per cm of root)	Intraradical infection Frequency (%)	Intraradical infection Intensity (%)
5	R	0.15 a	0	n.d	0	0
	RM	0.18 a	0	n.d	0	0
10	R	0.40 ab	0	n.d	0	0
	RM	0.43 ab	0	n.d	39 a	6 a
15	R	0.92 abc	5 a	n.d	0	0
	RM	0.89 abc	6 a	n.d	87 b	41 b
20	R	1.33 cd	6 a	0.00	0	0
	RM	1.15 bc	35 bc	2.80 a	87 b	42 b
25	R	2.13 d	15 ab	0.00	0	0
	RM	2.05 d	97 de	5.10 b	92 b	42 b
30	R	3.33 ef	42 bc	n.d	0	0
	RM	3.03 e	121 ef	n.d	92 b	53 b
35	R	3.13 ef	33 bc	0.00	0	0
	RM	3.93 fg	140 ef	6.10 b	100 b	70 c
40	R	4.48 g	63 cd	n.d	0	0
	RM	5.28 h	158 f	n.d	100 b	70 c
45	R	3.65 ef	60 bcd	0.00	0	0
	RM	6.48 i	120 ef	6.07 b	100 b	75 c
50	R	5.75 hi	55 bcd	n.d	0	0
	RM	8.05 j	121 ef	n.d	100 b	75 c

n.d: non etermined; R: inoculation with *Rhizobium*; RM: double inoculation with *Rhizobium* and *Glomus moseae*. Values followed by same letter within columns do not differ significantly, P = 0.01 Duncan, 1955).

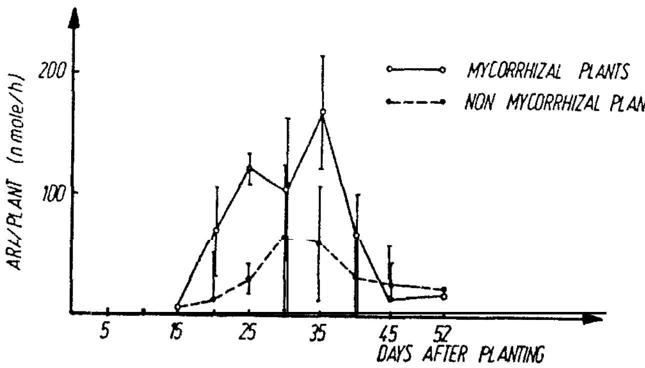

Figure 1. Time course of acetylene reduction activity (ARA) expressed in nanomoles C_2H_4 per plant per h in mycorrhizal and non-mycorrhizal cowpeas.

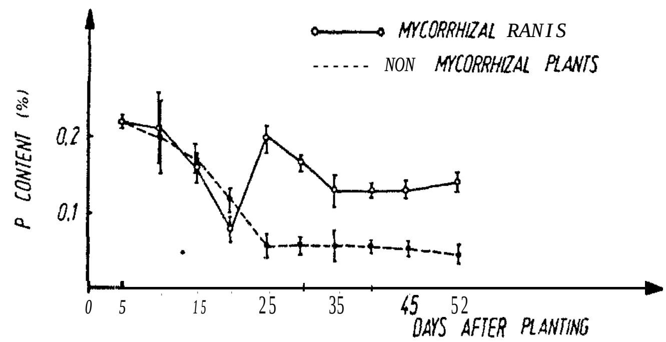

Figure 2. Time course of P content (%) of shoots in mycorrhizal and non-mycorrhizal cowpeas.

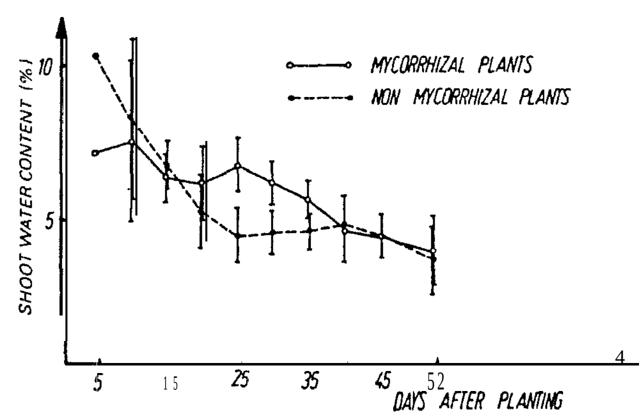

Figure 3. Time course of water content of shoots in mycorrhizal and non-mycorrhizal cowpeas.

P and water content of shoots. Fig. 2 shows that the time course of P content (P %) in mycorrhizal plants differed strikingly from that of non-mycorrhizal plants. In the non-mycorrhizal plants shoot P % declined for 25 days after planting, then reached a low level at which it remained up to day 50. Instead of these two phases, the time course of mycorrhizal plants exhibited a very different pattern with three phases:

- a first phase (day 0 to 20) during which P % decreased;
- a second phase (day 20 to 25) during which P % rapidly increased, this enrichment phase being concomitant and probably related to the extention of extraradical hyphae;
- a third phase (day 20 to 50) of moderate decline of P %, followed by a stabilization of P % at a relatively high level.

Fig. 3 shows that, during the time interval between day 20 and 30, the water content in mycorrhizal plants was significantly higher than that of non-mycorrhizal plants. The higher water content during this period is probably related to the concurrent enhancement of P inflow, if we consider, as a number of authors (e.g. SAFIR *et al.*, 1972; LEVY and KRIKUN, 1980) have, that water uptake and transfer are facilitatcd by the improvement of P nutrition. We have already mentioned thar the enhancement of P inflow was probably the consequence of the extension of the netwnrk of extraradical hyphae of *Glomus mosseae*.

DISCUSSION

The data presented here suggests that VA mycorrhizal infection proceeds in three steps:

- a first step during which the roots are progressively infected, the level of infection being measured by our "frequency" index, which approximates the "infected root length" index used by a number of authors;
- a second step during which the hyphae pervade the root cortex, the intensity of this process being measured by our "intensity" index, which indicates the percentage of the cortex volume invaded by the hyphae and not rhe root infected length. Our intensity index would approximate the "intraradical biomass" as defined by BETHLENFALVAY *et al.* (1982);
- a third step during which the VA fungus develops its extraradical hyphae throughout the soil and around the roots.

One should be aware of the fact that this succession of events cannot be generalized since there are probably large variations according to the environmenral conditions and to the specific characteristics of the partners of the symbiosis. However. there is one point on which most authors agree, at least in theory. that the extraradical hyphae are essential in promoting the growth of mycorrhizal plants (TINKER, 1980). Thus it is surprising that so few papers have dealt with the evaluation of extraradical hyphae. The main reason is probably that this type of assessment is very difficult.

A few techniques have been proposed. In addition to the one Presented here, let us quote the following:

- evaluation of the chitin content of the rooting medium (BETHLENFALVAY *et al.*, 1982);
- microscopic measurement of hyphae extracted from the rhizosphere soil or from the roots (KUCEY and PAUL, 1982);

- weighing the hyphae collected by stripping the roots and wet-sieving the rhizosphere soil (SANDERS *et al.*, 1977).

Since these methods are time consuming, it is tempting to use the regression analysis approach to obtain correlation between the amount of extraradical hyphae and any other parameter whose assessment would be easier (e.g. KUCEY and PAUL, 1982). However, even if a relationship is shown to be relevant in a given situationit is not necessarily applicable in other circumstances.Thus SANDERS et *al.*(1977)found that the dry weights of extraradical hyphae were proportional to the total infected root length in their experiment on onion. The results of the study we present here show that such a relation is not appropriate. Consequently it is advisable to assess the amount of extraradical hyphae using direct methods.

It is interesting to compare the amount of extraradical hyphae as calculated by differents authors. If this characteristic is expressed as cm of hyphae per cm of root, the figures published range between 80 cm (SANDERS and TINKER, 1973) and 1.5 cm (SANDERS et *al.*, 1977). Thus the figure obtained in our experiment falls within this range, even though it must be an underestimation as all the extraradical mycelium cannot be recovered by the method used. Nevertheless,our data supports the idea that extraradical hyphae play a major role by promoting the uptake of P and consequently improving the water transport to the plant and markedly enhancing N_2 fixation in the case of N_2-fixing plants. However,we have seen that there may be a delay between the Initiation of these processes and the initiation of the stimulation of the plant growth itself, which indicates that other phenomena may interfere, such as the C and/or P drain by the endophytes.

Finally it appears that thr study of VA fungal infection and of its consequences on the host should be carried out over a period, if possible throughout a growth cycle, in order to give a sufficient insight into the symbiotic system.

BIBLIOGRAPHY

BETHLENFALVAY G.J., PACOVSKY R.S. and BROWN M.S. 1982. *Phytopathology*, *72*, 894-897.

GUEYE M., DIEM H.G. and DOMMERGUES Y.R. 1985. *Mircen* J. (in press).

KUCEY R.M.N. and PAUL E.A. 1982. *Soil Biol. Biochem.*, 14, 413-414.

LEVY Y. and KRIKUN J. 1980. New *Phytol.*, *85*, 25-31.

OLLIVIER B., BERTHEAU Y., DIEM H.G. and GIANINAZZI-PEARSON V. 1983. *Can. J.* Bot., 61, 354-358.

SAFIR G.R., BOYER J.S. and GERDEMAN J.W. 1972. *Plant Physiol.*, *49*,

SANDERS F.E. and TINKER P.B. 1973. *Pestic. Sci.*, *4*, *385-395.*

SANDERS F.E., TINKER P.B., BLACK R.L.B. and PALMERLY S.M. 1977. *New Phytol.*, *78, 257-268.*

TINKER P.B. 1980. In: The role of phosphorus in agriculture, 617-654 (F.E. KAMPRATH *et al.* eds) *American Society* of *Agronomy*,