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Full Length Research Paper

Growth and *in vitro* phosphate solubilising ability of *Scleroderma sinnamariense*: A tropical mycorrhiza fungus isolated from *Gnetum africanum* ectomycorrhiza root tips

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The growth and ability of *Scleroderma sinnamariense* isolated from *Gnetum africanum* ectomycorrhiza root tips to solubilise calcium tetrahydrogen diorthophosphate, calcium phytate, hydroxyapatite and amorphous iron phosphate was assessed in axenic culture under a range of conditions. *S. sinnamariense* grew on all P sources when NH_4^+ was sole nitrogen source, but failed to grow on amorphous iron phosphate in unbuffered media with peptone as sole nitrogen source. The fungus solubilised calcium tetrahydrogen diorthophosphate and hydroxyapatite in unbuffered media with NH_4^+ as nitrogen source. The internal phosphorus status of the mycelia had no influence on the ability of the fungus to solubilise the substrates. The data confirmed that mycorrhizal fungi can access P sources which are normally unavailable and may pass on the absorbed P to the plant partner in case of symbiosis.

Key words: Phosphorus solubilisation, *Scleroderma sinnamariense,* mycorrhiza fungus.

INTRODUCTION

Phosphorus (P) is the most likely element to limit plant growth, next to nitrogen. This is because a large proportion of P in the soil is poorly soluble and most of the P is rapidly fixed in soil into fractions that are unavailable to plant roots (Sanyal and De Datta, 1991). Phosphorus is present in soil in many different physical and chemical forms, which include fluorapatite, hydroxyapatite, oxyapatite, chlorapatite, wavellite, monetite and inositol phosphates (Lapeyrie et al., 1991). In most acid soils like the type found in Cameroon, most P is deposited as various forms of amorphous and crystalline iron and aluminium phosphates (Sanyal and De Datta, 1991). Plants have developed differing physiological mechanisms and strategies for acquiring P from soil, with mycorrhiza formation being one of the most widespread ways of overcoming P deficiency by land plants. There is increasing evidence that mycorrhizal plants may be able to use soil P fractions held in specific physico-chemical forms that are otherwise unavailable to uncolonised plants (Koide and Kabir, 2000; Smith et al., 2000; Tibbett and Sanders, 2002; Smith and Read, 2008). This may be achieved by mycelia extending beyond the depletion zone, thereby increasing surface area for absorption. It may also be possible via the production of acids, which solubilize the phosphate making it available for absorption.

In Cameroon *Gnetum* spp. grow principally in tropical rain forests in mixed communities supported by acid oxisols and ultisols characterised by low soluble inorganic P. This leads to competition for limited nutrients such as P. Transformation of P derived from organic matter would be paramount in the P nutrition of such plants, as well as in nutrient cycling in such ecosystems (Johnson et al., 2003). Some researchers have suggested that the release of organic acids from EM fungi may solubilise recalcitrant mineral phosphates (Leyval and Berthelin, 1986; Lapeyrie et al., 1991). Others believe that before insoluble organic P can be broken down by fungal phosphatases and the resulting phosphate absorbed by the roots, they would firstly have to be solubilised (Lapeyrie et al., 1991). There is therefore a necessity to evaluate the solubilisation abilities of fungi *in vitro*.

The main objective of the work reported here was to evaluate the ability of *Scleroderma sinnamariense* to solubilise P and grow in the presence of different sparingly soluble phosphorus sources with either peptone or ammonium as the N source. The effect of buffering on growth and solubilisation was also assessed. *Pisolithus tinctorius* was included for comparison.

MATERIALS AND METHODS

The method used here was reported by Lapeyrie et al. (1991) and by Van Leerdam et al. (2001).

Fungal culture

S. sinnamariense was isolated from *Gnetum* roots and identified using molecular techniques as described in Bechem (2004). One set of starter cultures for this experiment had been grown for 30 days on modified Melin-Nokran (MMN) agar with ammonium-N; another set was grown on MMN agar with peptone-N, whilst the last set was grown on MMN agar with ammonium-N in absence of a phosphorus source. This was to check for the influence of internal mycelia P status and N source on solubilisation.

Basal medium

The basal medium consisted of $MgSO_4.7H_20$ 140 mg/L, $CaCl_2$ 50 mg/L, $ZnS0_4$ 3 mg/L, FeEDTA mg/L, thiamine HCl 10 µg/L and agar 10 g/L, as modified in this study from Marx (1969).

Nitrogen sources

The nitrogen sources were $(NH_4)_2SO_4$ and peptone. When $(NH_4)_2SO_4$ was added at 0.284 g/L, glucose was 3.004 g/L. Peptone was added at 0.380 g/L with a corresponding glucose amount of 2.100 g/L. This gave a C:N ratio of approximately 20:1 in both cases.

P sources

The P sources used were calcium tetra hydrogen di-orthophosphate ($CaH_4(PO_4)_2$) (BDH®), calcium phytate ($C_6H_6Ca_6O_{24}P_6$) (Sigma®), hydroxyapatite ($Ca_5(PO_4)_3OH$) (Sigma®) and amorphous iron phosphate. Hereafter, these P sources would be referred to as $CaPO_4$, phytate, apatite and $FePO_4$ respectively. These P sources were chosen because most inorganic P in soils occurred in these forms (Richardson, 2001) with iron and aluminium phosphates being predominant in tropical acid soils (Sanyal and De Datta, 1991; Bolan, 1991). The preparation of the amorphous iron phosphate was based on the method first reported by Carter and Hartshorne (1923) and subsequently used by Cate et al. (1959) and Bolan et al. (1987) for the preparation of amorphous and eventually crystalline iron phosphate.

In the preparation process, 22.5 g of carbonyl iron powder was dissolved in reagent-grade orthophosphoric acid (270 ml of 50% solution). The solution was diluted to 600 ml, filtered and the iron content oxidised by addition of 30% hydrogen peroxide. Diluting the solution to 25 L precipitated amorphous ferric phosphate. The precipitate was washed several times using deionised water, followed by decantation and vacuum filtration between successive washings. The appearance of faint brownish-yellow coloration on the precipitate was assumed to indicate that the phosphorus-iron ratio had attained unity (Carter and Hartshorne, 1923). The precipitate was then allowed to dry at room temperature before storage in a dessicator. The resulting powder was used in the assay as amorphous iron phosphate.

Preparation of P sources, culture media and inoculation

Distilled water was added to 5 g of each P source and autoclaved for 15 min at 121°C. Following cooling, contents were filtered through a 0.45 µm Millipore filter followed by several washes in sterile distilled water. This was done under sterile conditions. For each N and P source, one batch was buffered with 2- (N-morpholino) ethane-sulphonic acid (MES) at 50 mM (Van Leerdam et al., 2001), whilst the other was unbuffered. This was to check for the effect of pH on solubilisation. The pH of the medium was adjusted to 5.5 prior to autoclaving at 121°C for 15 min. Peptone was filter sterilised and added to an already autoclaved medium. Basal medium was then poured into a 9 cm diam. Petri dish to give a thin layer. Each previously autoclaved P sources was then added to a portion of basal medium resulting in a suspension, which was poured into the plates on top of the first layer of agar and allowed to solidify. Each dish was inoculated with a 5 mm diam fungal plug cut from the edge of an actively growing mycelial mat. Each phosphate, nitrogen and buffer treatment was done in three replicates for each inorganic phosphorus (Pi) starved or Pi sufficient isolate. Incubation was at 30°C in the dark. The mycelial, media, P source and buffer combinations used in the assay are shown in Table 1.

Plates were observed visually every 10 days for zones of clearing around the mycelial mat. Plates, which showed no zones of clearing, were observed using a dissecting and compound microscope to look for zones of clearing which might be smaller than the fungal colony. The diameter of fungal colony and that of zone of clearing was measured by taking the longest axis of fungal colony and any cleared ring at the end of the experiment. The experiment ran for 23 days; however, the plates were checked again after 60 days of incubation to see if there was any change in solubilisation with time.

Statistical analysis

Data were analysed using the General Linear Model of Minitab 13. Three-way ANOVA was used to test for the effect of P sources ($CaPO_4$, $FePO_4$, Apatite), internal mycelia P status and buffer on colony diameter (growth). A two-way ANOVA to evaluate the effect of inoculum P status and P sources ($CaPO_4$, $FePO_4$, Apatite, phytate) on colony diameter in absence of buffer was also run.

RESULTS

Growth of fungi

Generally, colony diameter ranged from 5 mm to a maximum of 71 mm. Growth was considered to have taken place when the colony diameter at the end of the experiment was greater than 5 mm.

Growth of fungi on peptone-N

P sufficient *Sclerodema* showed growth on all P sources except on $FePO_4$ in unbuffered media (Figure 1a). The

Table 1. Inoculum status, assay medium, P source and buffer combinations used in the solubilisation experiment.

Internal mycelial N/P status	Media N/P status	P source	Presence/absence of buffer
Peptone + P	Peptone + P	$CaPO_4$	MES
Peptone + P	Peptone + P	$CaPO_4$	No MES
Peptone + P	Peptone + P	Phytate	MES
Peptone + P	Peptone + P	Apatite	MES
Peptone + P	Peptone + P	Apatite	No MES
Peptone + P	Peptone + P	$FePO_4$	MES
Peptone + P	Peptone + P	$FePO_4$	No MES
Ammonium + P	Ammonium + P	$CaPO_4$	MES
Ammonium + P	Ammonium + P	$CaPO_4$	No MES
Ammonium + P	Ammonium + P	Phytate	MES
Ammonium + P	Ammonium + P	Apatite	MES
Ammonium + P	Ammonium + P	Apatite	No MES
Ammonium + P	Ammonium + P	$FePO_4$	MES
Ammonium + P	Ammonium + P	$FePO_4$	No MES
Ammonium - P	Ammonium + P	$CaPO_4$	MES
Ammonium - P	Ammonium + P	$CaPO_4$	No MES
Ammonium - P	Ammonium + P	Phytate	MES
Ammonium - P	Ammonium + P	Apatite	MES
Ammonium - P	Ammonium + P	Apatite	No MES
Ammonium - P	Ammonium + P	$FePO_4$	MES
Ammonium - P	Ammonium + P	$FePO_4$	No MES

source of medium P, the presence of buffer and the interaction of these two factors had significant effects ($P<0.001$) on colony diameter (Table 2). Maximum growth of 40 mm was observed on $FePO_4$ in buffered conditions. Growth of fungus in buffered medium was superior to that observed in unbuffered medium. The difference was greatest for $FePO_4$ (Figure 1a). Growth of fungus on phytate in unbuffered medium was comparable to that on $CaPO_4$ and apatite but better than that on $FePO_4$ (Figure 1a). P sufficient *Pisolithus* also showed growth on all P sources in both buffered and unbuffered media (Figure 1b) except when grown on Ca phytate in unbuffered medium.

The source of P had a significant effect ($P<0.001$) on growth. Maximum growth of 62 mm was observed following growth of fungus on $CaPO_4$ in buffered medium. Growth of the fungus on $CaPO_4$ and $FePO_4$ in buffered medium was superior to that in unbuffered medium on these same P sources. However, growth on apatite in unbuffered medium was superior to that observed in buffered medium (Figure 1b). The effect on growth of the interaction between the buffer and P source was also significant at $P<0.05$. Growth of the fungus on $CaPO_4$ was comparable to that in apatite in unbuffered medium but superior to that observed on $FePO_4$ and phytate (Figure 1b). A summary of the statistical analysis of data from this experiment is presented on Table 2.

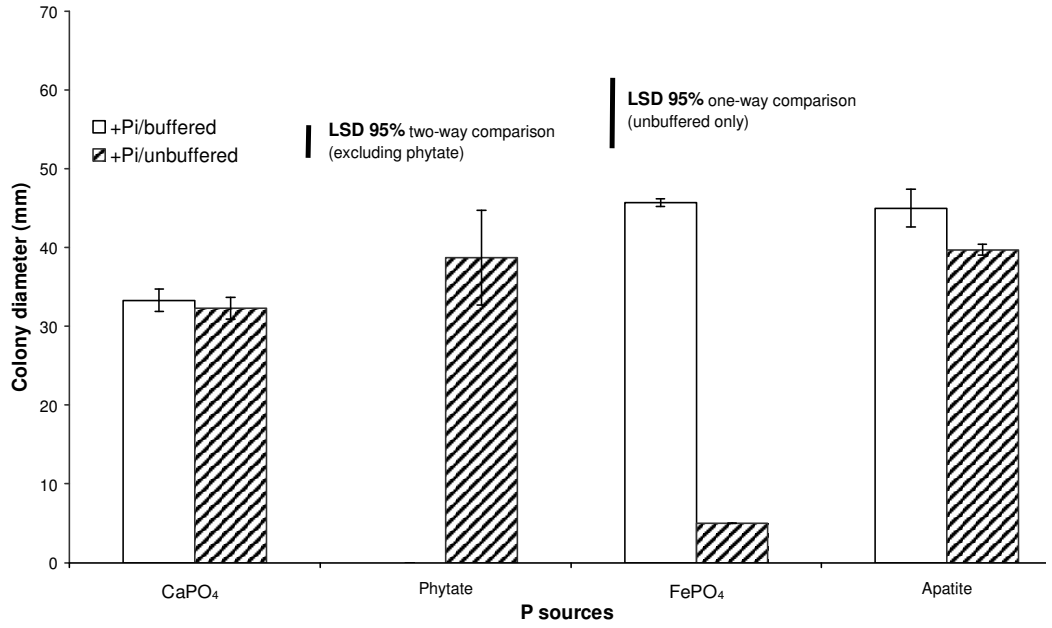
Growth of fungi on ammonium-N

Scleroderma

The fungus grew on all P sources but growth on $CaPO_4$ and apatite was better than growth on $FePO_4$ (Figure 2a). P sufficient inoculum grew better in comparison to P starved inoculum on $CaPO_4$ and apatite but not on $FePO_4$. Growth on buffered medium by the fungus was better than growth on medium devoid of buffer and the difference was greatest for $FePO_4$ (Figure 2a). Growth on phytate in unbuffered medium was comparable to that on apatite and $CaPO_4$ and better than that on $FePO_4$ (Figure 2b).

Pisolithus

The fungus grew on all P sources except when P sufficient fungus was assayed on $FePO_4$. P sufficient inoculum showed better growth in comparison to P starved inoculum but the difference was not significant (Table 3). Growth of the fungus on apatite in unbuffered medium was superior to growth in buffered medium whereas on $CaPO_4$, growth in buffered medium was greater than that in unbuffered medium (Figure 3a). The fungus was unable to grow on phytate in unbuffered medium, an observation, which was similar to that of P



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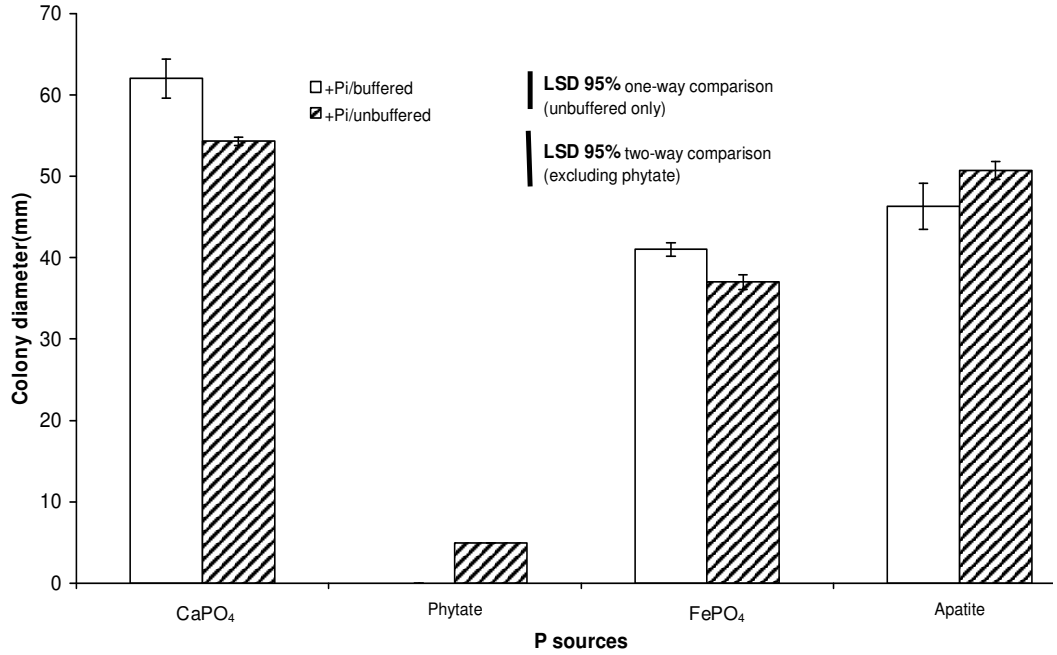

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Figure 1. Growth (mm diameter) variation of *S. sinnamariense* (a) and *P. tinctorius* (b) on different sparingly soluble P sources with peptone-N in the presence or absence of a buffer. The + Pi in the legend represents the internal Pi status of mycelia inoculum. Each value is a mean of three replicates. Vertical bars represent standard error of the mean. $CaP0_4$, Phytate, $FePO_4$ and Apatite on the X-axis represent Calcium tetrahydrogen diorthophosphate, calcium phytate, amorphous iron phosphate and hydroxyapatite respectively.

sufficient fungus on $FePO_4$ (Figure 3b). P starved fungus showed a growth pattern on $FePO_4$ which was unusual (Figure 4). A summary of the statistical analysis of data are shown in Tables 3 and 4.

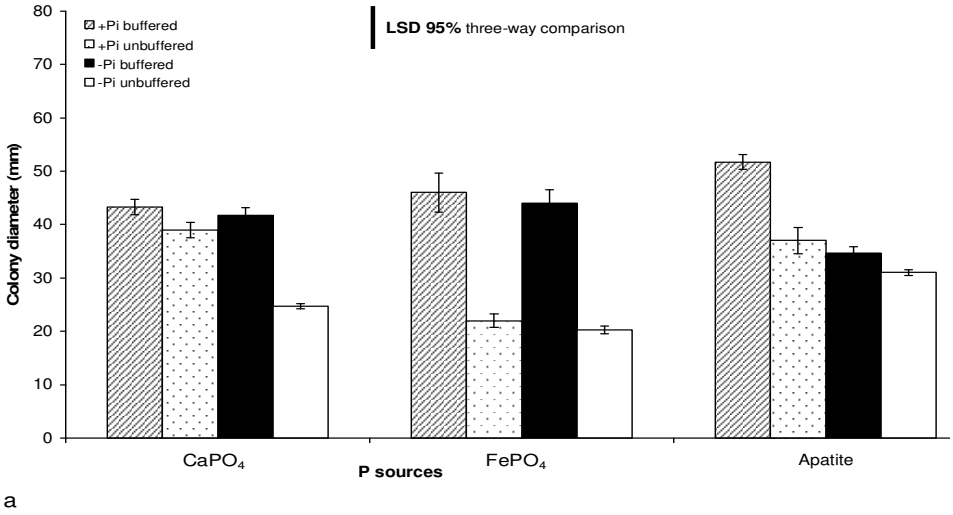
Solubilisation of sparingly soluble P sources

The results of solubilisation by both *Scleroderma* and *Pisolithus* of the different P sources and in the presence

Table 2. Summary of two-way analysis of variance of the effects of buffer and P source on growth of Scleroderma and Pisolithus on MMN containing peptone-N. Starter inoculum was P sufficient.

Species	Source of variation	DF	F	P
Scleroderma	Buffer	1	80.28	* * *
	P sources	2	52.97	* * *
	Buffer* P source	2	113.40	* * *
Pisolithus	Buffer	1	1.88	ns
	P sources	2	38.45	* * *
	Buffer* P source	2	3.96	*

n = 3, *P<0.05, ** P<0.01, *** P<0.001. ns: not significant. P sources are $CaPO_4$, $FePO_4$, Apatite.



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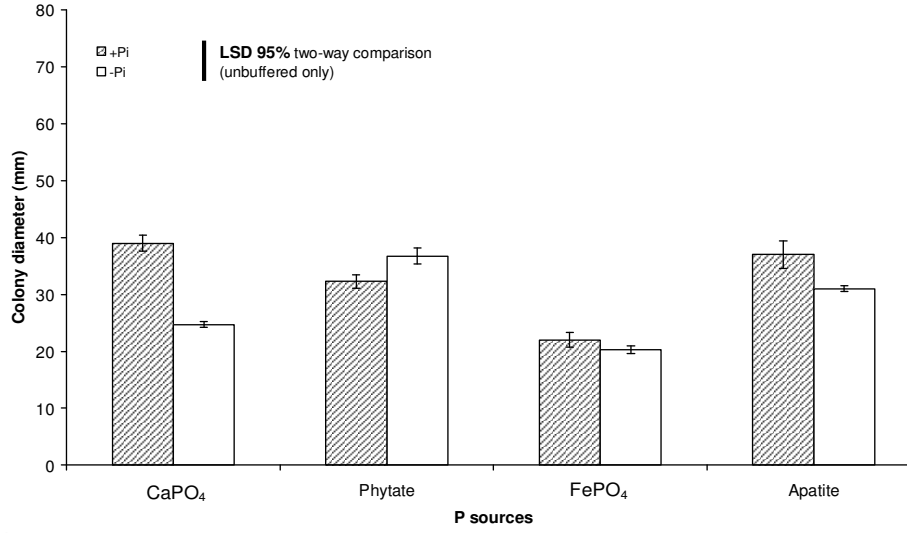

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Figure 2 Growth of *Scleroderma* on different sparingly soluble P sources in MMN containing ammonium-N. (a) Growth on buffered and unbuffered medium. (b) Growth on unbuffered medium only. Each value is a mean of three replicates. Vertical bars represent standard error of mean. P in legend represents the internal P status of inoculum. CaP04, Phytate, FePO4 and Apatite on the X-axis represent Calcium tetrahydrogen diorthophosphate, calcium phytate, amorphous iron phosphate and hydroxyapatite respectively.

Table 3. Summary of three-way analysis of variance of the effects of inoculum P status, presence or absence of buffer in growth medium and source of inorganic P, on colony diameter following growth of *Scleroderma* and *Pisolithus* for 23 days on MMN agar medium containing sparingly soluble P sources.

Species	Source of variation	DF	F	P
Scleroderma	P source	2	7.29	0.003**
	Inoculum	1	33.92	0.000***
	Buffer	1	142.12	0.000***
	Inoculum*Buffer	1	0.03	0.857^{ns}
	Inoculum*P source	2	5.36	0.012*
	Buffer*P source	2	14.56	0.000***
	Inoculum*Buffer*Psource	2	7.85	0.002**
Pisolithus	P source	2	69.16	0.000***
	Inoculum	1	1.89	0.182^{ns}
	Buffer	1	102.83	0.000***
	Inoculum*Buffer	1	0.00	1.000ns
	Inoculum*P source	2	9.68	0.001***
	Buffer*P source	2	81.25	0.000***
	Inoculum*Buffer*Psource	2	4.75	0.018*

P sources are $CaPO_4$, $FePO_4$, Apatite.

of NH_4^+-N are shown in Table 5. Cleared zones were observed in two cases for *Scleroderma* (Figure 5) but none was observed with *Pisolithus*. The mean diameter of solubilisation rings are shown in Figure 6. There was solubilisation of $CaPO_4$ and hydroxyapatite by both P starved and P sufficient *Scleroderma*. Solubilisation was only possible when N source of medium was ammonium and in the absence of a buffer. Maximum solubilisation ring diameter of 38 mm was recorded on hydroxyapatite, by the P starved fungus. P starved fungus demonstrated an ability to solubilise apatite and $CaPO_4$ which was superior to that shown by P sufficient fungus. This difference was greatest for apatite. Solubilisation of $CaPO_4$ by P sufficient fungus was comparable to solubilisation of apatite. When expressed as a percentage of fungal colony diameter, solubilisation by P starved fungus of hydroxyapatite was 119 and 128 for $CaPO_4$, as calculated from Figures 2 and 6 using the following equation:

Ring diameter ÷ colony diameter × 100% ----------(1)

Pisolithus on the other hand was unable to solubilise any of the P sources in both buffered and unbuffered conditions.

DISCUSSION

Our observations in the solubilisation experiment are similar to those in investigations using ericoid and other EM fungi species. In the study carried out by Van Leerdam et al. (2001) the *Hymenoscyphus* isolates only solubilised hydroxyapatite in the presence of ammonium-N and in unbuffered medium. This observation, quite similar to that of *Scleroderma* isolate in this study, is probably due to proton expulsion. The resulting pH change in the medium might then lead to the observed solubilisation. This phenomenon is confirmed by studies showing that during ammonium transport by the hyphae, there is H^+ secretion into the medium (Jennings, 1997). Some fungi like *Paxillus involutus* have been shown to produce oxalic acid during growth on nitrate-N (Lapeyrie et al., 1987). It is thought that such acid helps in P solubilisation by acidification (Jones et al., 1980). So far, no study had looked at solubilisation on N sources other than ammonium and nitrate. In an assay on growth of these *Scleroderma* and *Pisolithus* isolates on various N sources including peptone, it was observed that growth on peptone was followed by a fall in pH of medium (Bechem, unpublished). But the resulting decrease in pH was not significant enough to bring about solubilisation by *Scleroderma* of hydroxyapatite and $CaPO_4$ in peptone-N as observed on ammonium-N. Nonetheless, the fact that both fungi did grow on these P sources means there was some solubilisation to permit growth. It is probable that an unknown proportion of the insoluble P provided must have dissolved, thus supporting growth without any apparent indication of solubilisation. It is unfortunate that other similar studies such as Van Leerdam et al. (2001) did not indicate whether growth was recorded in the absence of solubilisation or not.

There might be several reasons for the inability of the *Pisolithus* isolate to grow on Ca phytate in this study. One

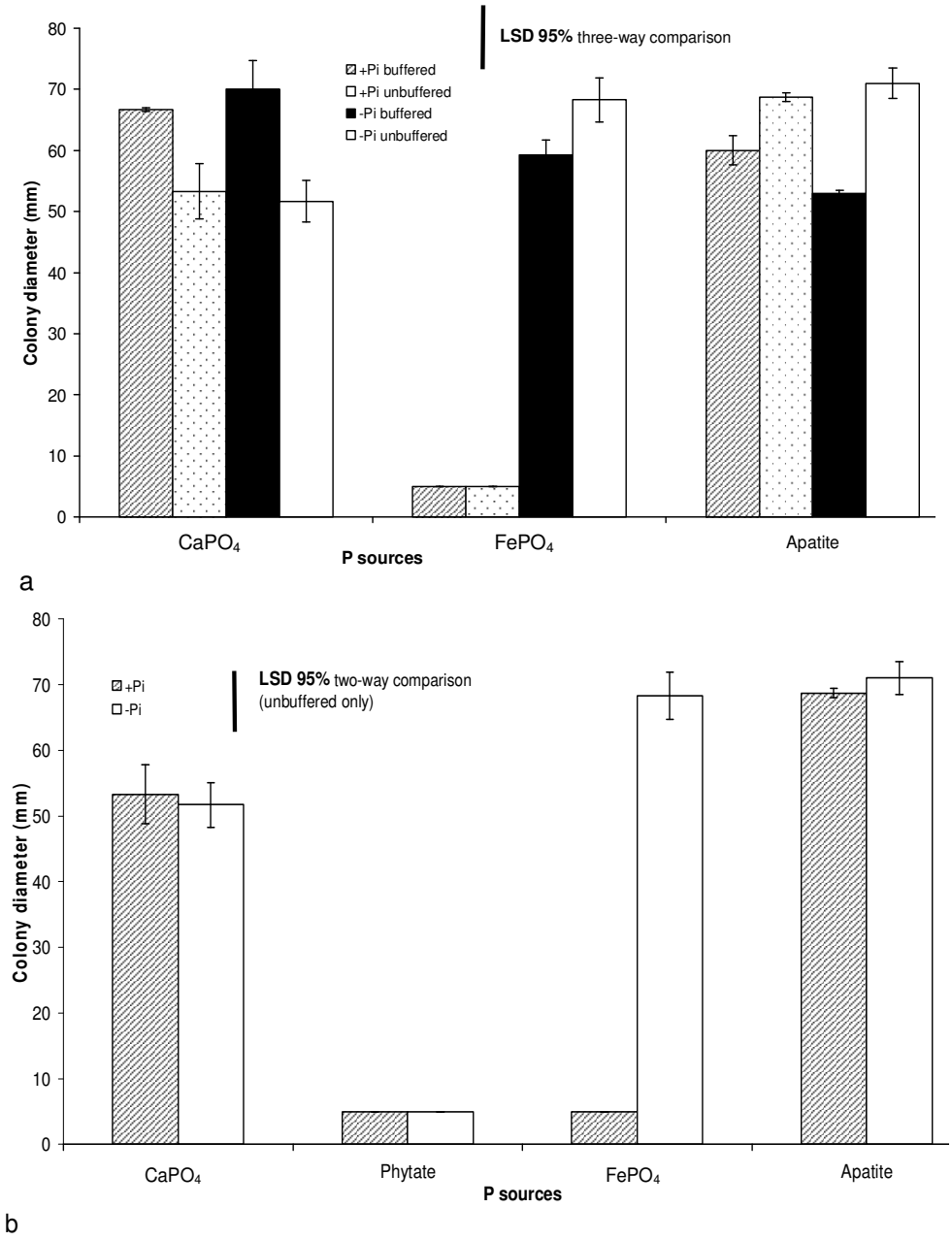

Figure 3. Growth of *Pisolithus* on different sparingly soluble P sources in MMN containing ammonium-N. (a) Growth on buffered and unbuffered medium. (b) Growth on unbuffered medium only. Each value is a mean of three replicates. Vertical bars represent standard error of mean. P in legend represents the internal P status of inoculum. $CaP0_4$, Phytate, $FePO_4$ and Apatite on the X-axis represent Calcium tetrahydrogen diorthophosphate, calcium phytate, amorphous iron phosphate and hydroxyapatite respectively.

possibility is that the amount of calcium phytate used in this experiment may have been toxic to the fungus. The *Pisolithus* isolate used in this study might have failed to produce phosphatase under the experimental conditions and therefore could not utilise the available P source. Both *Pisolithus* isolates used by Lapeyrie et al. (1991) grew on Ca phytate although only Isolate II could solubilise this P source when ammonium-N was provided. In the present study, the absence of intracellular P might have induced changes in the type of compounds exuded by the *Pisolithus* isolate. This alteration may be the reason for the observed strange growth pattern of the P starved fungus on $FePO_4$. Such an observation suggested specific selective synthesis

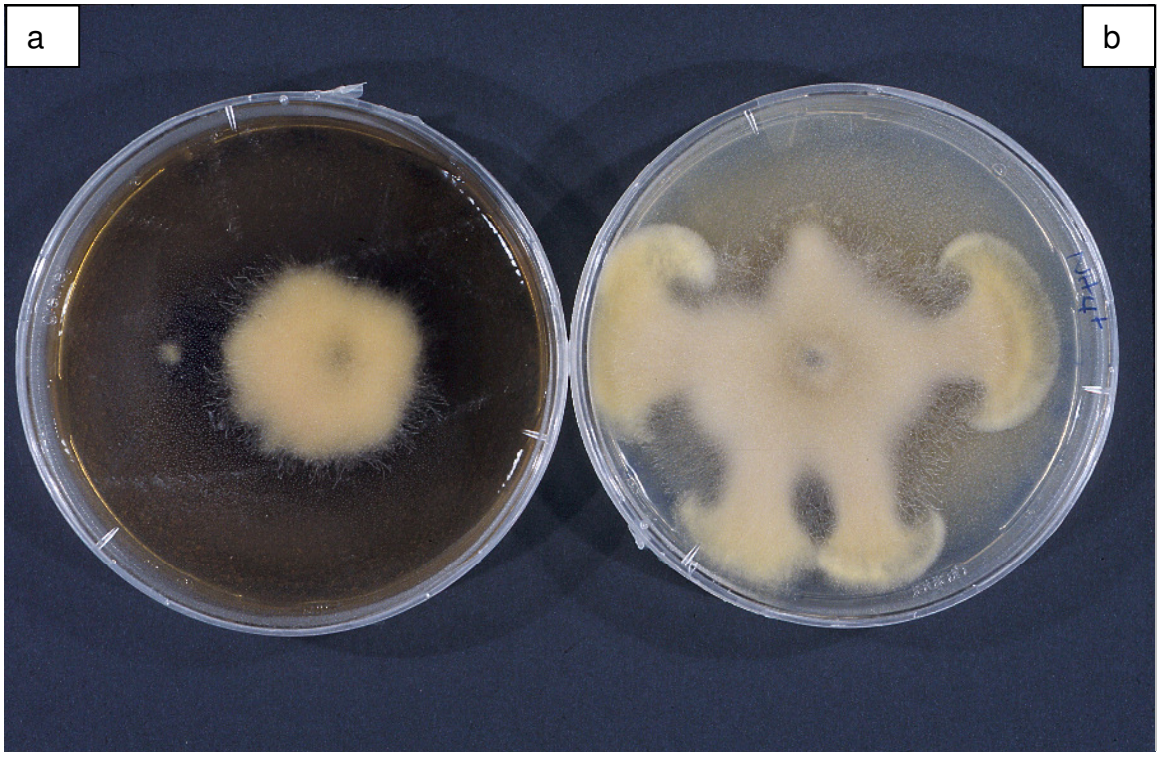

Figure 4. Growth of *Pisolithus* on nutrient agar. (a) Amorphous $FePO_4$ as opposed to the commonly observed radial growth pattern on MMN nutrient agar and (b) shows the unusual pattern observed with growth of fungus on MMN agar (minus potassium dihydrogen orthophosphate) containing ammonium-N.

Table 4. Summary of two-way analysis of variance of the effects of inoculum P status and source of inorganic P, on colony diameter following growth of Scleroderma and Pisolithus for 23 days on MMN agar medium containing sparingly soluble P sources in absence of buffer.

Species	Source of variation	DF	F	P
Scleroderma	P source	3	34.48	0.000***
	Inoculum	1	17.23	0.000***
	Inoculum*P source	3	13.62	0.000***
Pisolithus	P source	3	156.23	0.000***
	Inoculum	1	52.67	0.000***
	Inoculum*P source	3	51.35	0.000***

P sources are $CaPO_4$, $FePO_4$, Apatite, Phytate; ns: not significant; *** P<0.001, **P<0.01, *P<0.05.

and exudation of some compounds enabling the fungus to grow on $FePO_4$. The inability of *Scleroderma* and *Pisolithus* to solubilise some of the P sources assayed does not necessarily imply that they could not solubilise these P sources under natural conditions, with the help of some bacteria. Villegas and Fortin (2002) showed that the extramatrical mycelium of *Glomus intraradices* could solubilise sparingly soluble calcium phosphate *in vitro* only when grown in association with *Pseudomonas aeroginosa* and *Pseudomonas putida*. These bacteria were also unable to solubilise this P source without the help of the fungus. There is therefore a possibility that in association with some mycorrhiza helping bacteria, many more P sources could be accessed *in situ*.

It was also interesting to note that solubilisation of a P source did not necessarily mean better growth on that P source. In this study, it was noted within the same P source that, in situations where solubilisation occurred, the fungal growth diameter was inferior to that observed in the absence of solubilisation. A probable reason for such an observation may be that the fall in pH which is thought to have assisted in the solubilisation process demonstrated adverse effects on the fungal culture, causing a reduction in growth in comparison to the situation where solubilisation did not occur. Alternatively, the observed solubilisation of the P sources led to an increase in the amount of available P in the growth medium. The attained levels may eventually become toxic to the culture. Lower pH values are known to cause hydroxyapatite to dissolve and the protons react with the hydroxyl ion, as well as the phosphate groups in the lattice according to the mass balance as illustrated in Van Leerdam et al. (2001):

$$Ca_5(PO_4)_3OH(s) + 7H^+(aq) <=> 5Ca^{2+}(aq) + 3H_2PO_4^-(aq) \quad \text{----------(2)}$$

Table 5. Abilities of *Scleroderma* and *Pisolithus* to solubilise some organic and inorganic P sources in buffered and unbuffered media with ammonium as sole nitrogen source, after 23 days of growth.

Assay media		Inoculum pretreatment			
		Scleroderma sinnamariense		*Pisolithus tinctorius*	
		NH_4^+-N			
		+ Pi	- Pi	+ Pi	- Pi
NH_4^+-N $CaH_4(PO_4)_2$	Buffered	-	-	-	-
	Unbuffered	+	+	-	-
NH_4^+-N Ca phytate	Buffered	-	-	-	-
NH_4^+-N $FePO_4$	Buffered	-	-	-	-
	Unbuffered	-	-	-	-
NH_4^+-N hydroxyapatite	Buffered	-	-	-	-
	Unbuffered	+	+	-	-

+ Indicates zone of clearing around colony, **-** indicates no zone of clearing around colony. Consistent results were obtained in three replicate plates for each isolate.

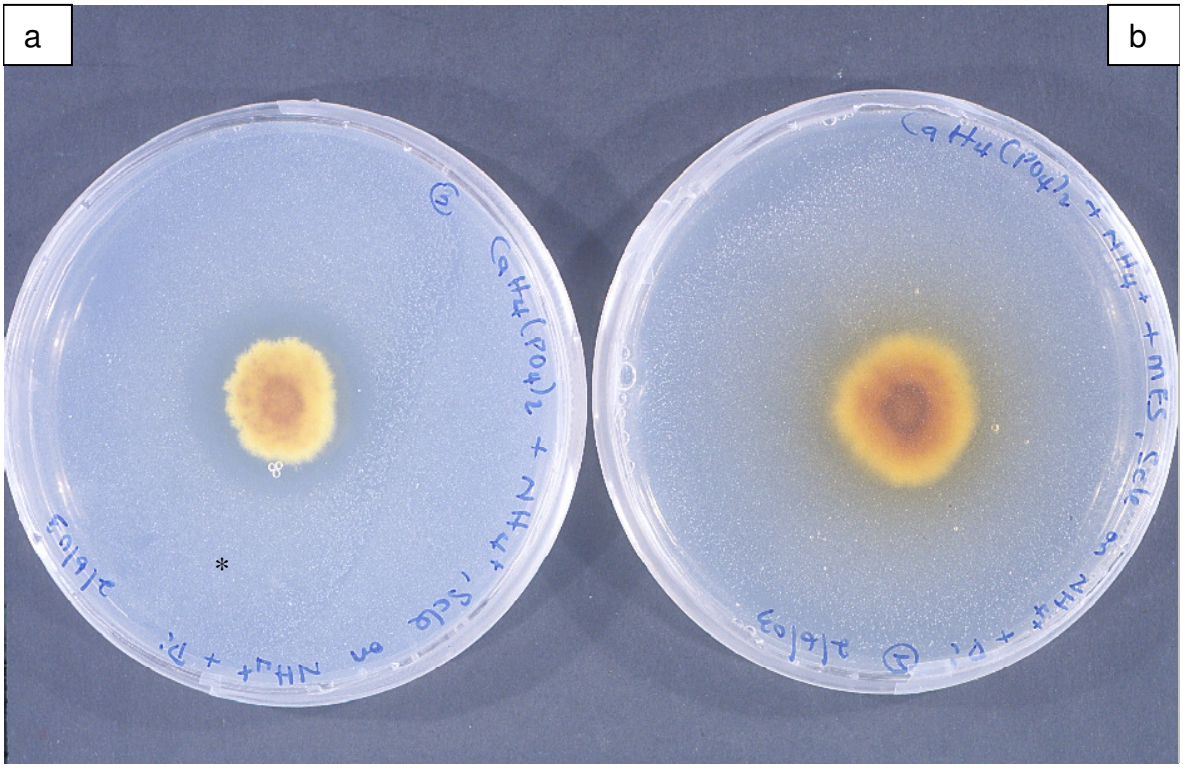

Figure 5. Growth and solubilisation of $CaPO_4$ in MMN agar medium by *Scleroderma*. (a). Solubilisation in unbuffered media. Asterik shows zone of clearing. (b). Inability of fungus to solubilise the same P source in presence of buffer.

Organic acids produced during growth of fungi may chelate the associated cations such as Ca^{2+} with a resulting enhancement in solubilisation (Attiwill and Leeper, 1987). Fungi have been shown to excrete a range of organic acids, including oxalic, citric and gluconic acids, during growth and the ability of fungi to solubilise sparingly soluble phosphates in the presence of NO^-_3 nitrogen has been attributed to the acidifying and chelating abilities of these compounds (Lapeyrie et al., 1991).

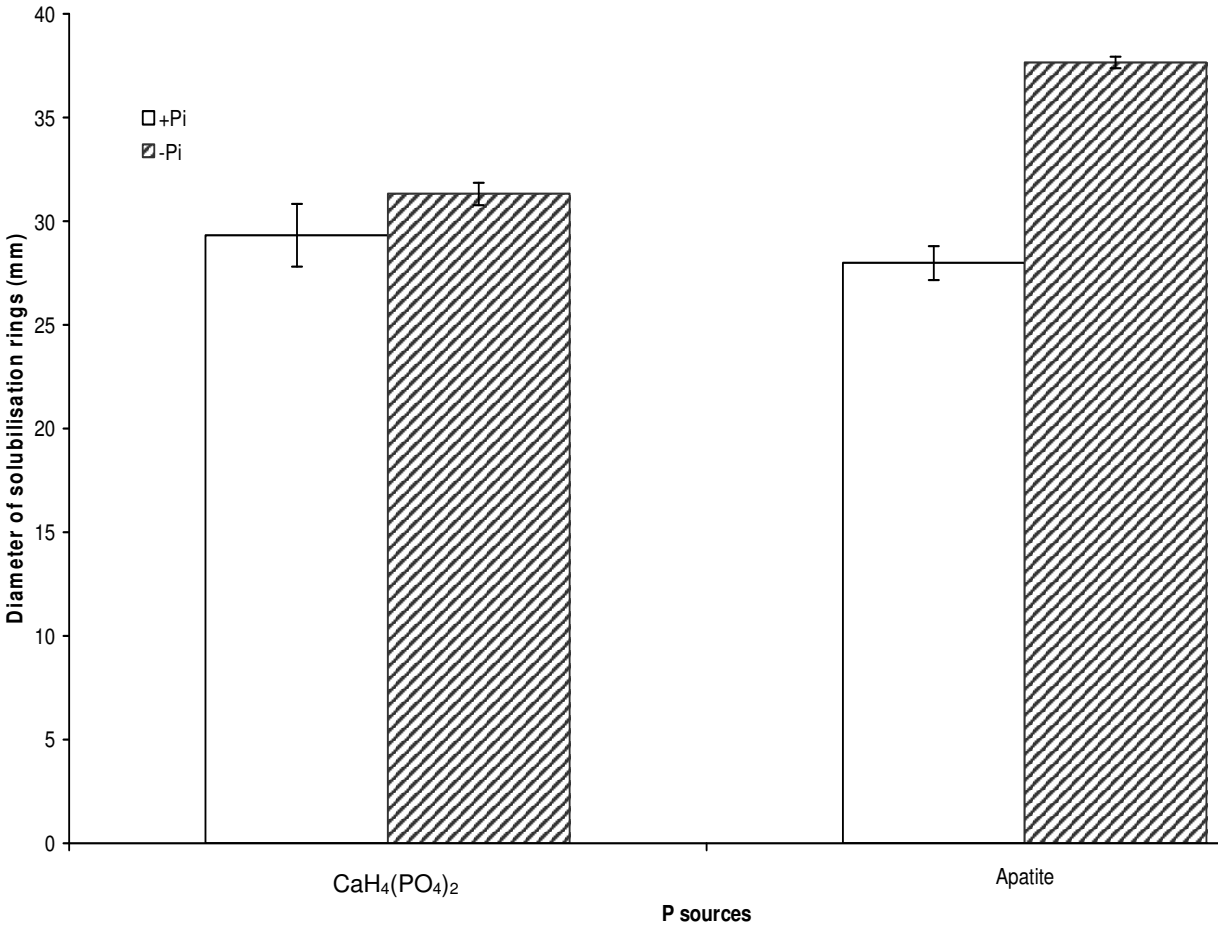

Figure 6. Variation in mean diameter (mm) of solubilisation rings following 23 days of growth of *Scleroderma* on MMN agar with insoluble hydroxyapatite and calcium hydrogen phosphate as only P source. Each value is a mean of three replicates. Vertical bars represent standard error of the mean. $CaH_4(PO_4)_2$ and Apatite on the X-axis represent calcium tetrahydrogen diorthophosphate and hydroxyapatite respectively. ± P in the legend represents the internal phosphorus status of mycelia inoculum, +P means phosphorus sufficient, -P means phosphorus starved.

Nonetheless, the resulting chelated complexes may have an adverse effect on the growth of the fungi in pure culture. Cairney and Smith (1992) found that inoculum P status does influence hyphal absorption of soluble phosphate. This defined the scope of this study to investigate the extent to which this might influence the abilities of *Scleroderma* and *Pisolithus* to solubilise the P sources tested. The internal P status of fungus inoculum had an effect on the solubilisation of hydroxyapatite but not of $CaPO_4$. Van Leerdam et al. (2001) did not observe any effect of P status of inoculum on solubilisation in their study. Although they concluded that solubilisation activities were not dependent on internal phosphorus concentrations, we believe from our assay that this may vary with P source and with the fungal species. The fact that *Scleroderma* could solubilise hydroxyapatite and calcium tetrahydrogen di-orthophosphate *in vitro* does not necessarily imply that its plant partner can utilise these P sources *in situ*. It would be necessary to demonstrate that solubilisation of a P source leads to uptake and eventual translocation to the host plant. Such a demonstration confirms that the host plant can utilise these P sources via their fungal partners.

During mycorrhization, both the plant and fungus undergo some physiological and biochemical changes (Smith and Read, 2008). These might affect their ability to access phosphorus in different forms. Although *in vitro* experiments give an idea of the different potentials of the fungi, we still cannot conclude as to what happens in their natural environment. To be able to do this, it would be necessary to look at the ability of the fungus-plant association to access different P sources in conditions quite similar to those of their natural environment.

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REFERENCES

Attiwill PM, Leeper GW (1987). Forest soils and nutrient cycles. (Melbourne University Press: Melbourne).

Bechem EE (2004). Mycorrhizal Status of Gnetum spp. in Cameroon: evaluating diversity with a view to ameliorating domestication efforts. PhD thesis, University of Aberdeen, Scotland, UK.

Bolan NS (1991). A critical review on the role of mycorrhiza fungi in the uptake of phosphorus by plants. Plant and Soil, 134: 189-207.

Bolan NS, Robson AD, Barrow NJ (1987). Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. Plant and Soil, 99: 401-410.

Cairney JWG, Smith SE (1992). Influence of intracellular phosphorus concentration on phosphate absorption by the ectomycorrhizal basidiomycete Pisolithus tinctorius. Mycol. Res., 96(8): 673-676.

Carter SR, Hartshorne NH (1923). The system of ferric oxide-phosphoric acid-water. A new phosphate. J. Chem. Soc., 123: 2223-2233.

Cate WE, Huffman EO, Deming ME (1959). Preparation of crystalline ferric phosphates. Soil Sci., 88: 130-132.

Jennings DH (1997). The physiology of fungal nutrition. Cambridge University Press, Cambridge.

Johnson AH, Frizano J, Vann DR (2003). Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. Oecologia, 135: 487-499.

Jones D, Wilson MJ, Tait JM (1980). Weathering of a basalt by *Pertusaria corallina*. Lichenologist (Oxford), 12: 2770-289.

Koide RT, Kabir Z (2000). Extra radical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *The* New Phytologist, 148: 511-517.

Lapeyrie F, Ranger J, Vairelles D (1991). Phosphate-solubilising activity of ectomycorrhiza fungi *in vitro*. Can. J. Bot., 69: 342-346.

Lapeyrie F, Chilvers GA, Behm CA (1987). Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus*. New Phytologist, 106: 139-146.

Leyval C, Berthelin J (1986). Comparison between the utilization of phosphorus from insoluble mineral phosphates by ectomycorrhizal fungi and rhizobacteria. In: *Physiological and Genetical Aspects of Mycorrhizae* (ed. V.Gianinazzi-Pearson & S. Gianinazzi), pp. 340-345. INRA: Paris, France.

Marx DH (1969). The influence of ectotrophic mycorrhizal fungi on resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology, 59: 153-163.

Richardson AE (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol., 28: 897-906.

Sanyal SK, De Datta SK (1991). Chemistry of phosphorus transformations in soil. Adv. Soil Sci., 16: 1-20.

Smith FA, Jakobsen I, Smith SE (2000). Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago trunculata*. New Phytologist, **147**: 357-366.

Smith SE, Read DJ (2008). Mycorrhiza Symbiosis, 3rd. ed. London , Academic Press.

Tibbett M, Sanders FE (2002). Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. Ann. Bot., 89: 783-789.

Van Leerdam DM, Williams PA, Cairney JWG (2001). Phosphate – solubilising abilities of ericoid mycorrhizal endophytes of *Woollsia pungens* (Epacridaceae). Aust. J. Bot., 49: 75-80.

Villegas J, Fortin JA (2002). Phosphorus solubilisation and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NO^-_3 as nitrogen source. Can. J. Bot., 80(5): 571-576.