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Effect of carbon and nitrogen sources on *in vitro* growth of *Scleroderma sinnamariense* Mont., a pantropical ectomycorrhizal fungus

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ABSTRACT

The utilisation of a range of carbon sources in the presence of either ammonium or peptone as sole nitrogen source by *Scleroderma sinnamariense*, an ectomycorhiza fungus isolated from *Gnetum africanum* was compared with *Pisolithus tinctorius. Scleroderma* showed significant growth of 17 mg with glucose as carbon source in the presence of peptone-N, whereas growth on the same carbon source in presence of ammonium-N was 8.5 mg. On the contrary, the *Pisolithus* isolate showed poor growth of 4.5 mg in peptone-N even with glucose as sole carbon source whilst growth on ammonium-N in the same carbon source was 15 mg. Utilisation of glucose, sucrose and starch by both *Pisolithus* and *Scleroderma* in ammonium-N led to a decrease in pH of the medium from 5.0 to 3.4, 3.5 and 4.8 respectively for *Pisolithus* and 3.2, 3.8 and 4.8 respectively for *Scleroderma*, whilst incubation on cellulose caused an increase in pH from 5.0 to 5.2. The decrease in pH was significant for both isolates when the carbon source was sucrose or glucose. Incubation of both fungi, on all carbon sources except glucose, in presence of peptone-N led in an increase in pH.

Keywords: Ammonium-N, Peptone-N, carbon utilisation, *Pisolithus tinctorius*.

INTRODUCTION

Mycorrhiza formation helps increase access to minerals for the plant host and is therefore very important for the successful establishment of the plant (Smith and Read, 2008). Tropical forest soils have poor nutrient status with most of the nutrients being held up in the organic fraction of the soil. The ability of mycorrhiza fungi to use these stored nutrients is paramount to the survival of the plant host in such soil types.

There is little information on the functional role of *Scleroderma sinnamariense*. This fungus had been shown to increase the host plants access to minerals like phosphorus and nitrogen (Bechem and Alexander, 2011), it could solubilise insoluble phosphorus sources (Bechem, 2011) and it did express both extracellular and cell bound phosphatase activity (Bechem, in press). Most information on functional aspects of fungi is on temperate species implying that Cameroonian ectomycorrhiza fungi (EM), just like other African isolates are relatively poorly studied.

Mycorrhizal fungi may function in the transfer of carbon, nutrients or water between plants (Simard et al., 2002) and thereby affecting plant and fungal community

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dynamics. Photosynthate carbon has been shown to transfer from host plants to mycorrhizal hyphae within hours (Johnson et al., 2002) and this drives half of the belowground microbial activity, with the rest fueled by heterotrophic metabolism of dead organic matter (Högberg and Högberg, 2002). Plants invest photosynthate carbon in mycorrhizas (instead of building their own roots) because the small and profuse hyphae have 60 times more absorptive area than fine roots (Simard et al., 2002). Generally, as nutrient and water limitations increase, plants allocate more photosynthate to mycorrhizal hyphae to increase soil resource uptake.

In the culture of microorganisms, most artificial media have supplies of carbon in simple forms. Glucose is the most utilised C source; a reason why it is often used in growth media. But with the existence of other potential C sources in the rhizosphere and bulk soil, the ability of mycorrhizal fungi to access these alternative C sources in pure culture and in symbiosis has been of interest to mycorrhizologists (Hutchinson, 1990; Read and Perez-Moreno, 2003; Tu et al., 2006; Talbot et al., 2008). They suggested that such fungi can secrete relatively small amounts of cellulases, amylases and other extracellular carbohydrases. The ability to grow saprotrophically may be of significance where ectomycorrhizal hosts are rare. Different taxa of mycorrhiza fungi are now recognized as targeting different carbon sources, implying niche partitioning. This niche partitioning can help explain why such a dazzling diversity of fungi are involved in carbon and nutrient metabolism in soils (Hansen et al., 2008).

Palmer and Hacskaylo (1970) investigated the growth of six ectomycorrhizal fungal species in pure culture on single carbon sources. They found that 'starter' glucose might be important in enabling greater hydrolysis of more complex sugars, probably by inducing the formation of an adaptive enzyme (Cochrane, 1958). Lamb (1974) investigated the ability of 21 ectomycorrhizal fungi of conifers to utilize 26 carbon sources. He observed that in the absence of added glucose the fungi utilised between 12 and 21 carbon sources, with best growth on glucose, mannose, fructose, cellobiose, trehalose, sucrose, dextrin glycogen, starch and pectin. After glucose supplementation, the fungi showed adaptive growth on between 0 and 11 more carbon sources. In his study, *Pisolithus tinctorius* was amongst the few EM fungi that could utilise and grow adaptively on the largest number of carbon sources. The carbon sources used in the study included, amongst those already mentioned above, erythrose, erythritol, arabinose, ribose, xylose, rhamnose, galactose, sorbose, inositol, mannitol, sorbitol, maltose, lactose, raffinose, cellulose and inulin. However, Taber and Taber (1987) showed that *Pisolithus tinctorius* grew poorly on cellulose, starch, sucrose, lactose, fructose and pectin in comparison to non-mycorrhizal fungi. They also noted that carbon utilisation pattern changed with culture medium pH, with an enhancement of growth by fungi being recorded on disaccharides at pH 4.0-5.0. The addition of small amounts of glucose also promoted some growth on sucrose, fructose and cellulose.

Most mycorrhiza fungi are difficult to grow in pure culture and the vigour often declines with time. Screening EM fungi for their ability to use carbon sources other than hexoses may help in solving growth vigour problems encountered in mycorrhiza research (Palmer and Hacskaylo, 1970).

This paper describes the ability of *Scleroderma sinnamariense* to grow on different C sources in the presence of either ammonium-N or peptone-N. Presently, there is no published work on carbon utilisation by ectomycorrhiza fungi of the genus *Scleroderma. Pisolithus tinctorius* on the other hand had been widely studied in this respect, though with conflicting observations. In this study an isolate of *Pisolithus tinctorius* was included for comparison.

MATERIALS AND METHODS

Fungi isolates

The *Scleroderma sinnamariense* isolate used in this experiment was obtained from

Gnetum africanum root tips as reported in Bechem (2004). In the isolation process, EM root tips were collected washed in tap water, followed by surface sterilisation in 6% chlorine for 1 min. The sterile tips were rinsed in two washes of sterile de-ionised water and plated on modified Melin Norkran agar (Marx, 1969) containing benomyl (I µg/ml), chlortetracycline (30 µg/ml) and streptomycin (10 µg/ml).

Growth medium

The carbon sources used were glucose, sucrose, soluble starch, cellulose and cellulose with 'starter' glucose. The utilisation of these carbon sources was evaluated on two N sources: ammonium sulphate and peptone. The source of carbon and nitrogen, as well as the amounts added to basal medium are shown in Table 1. The amount of each carbon source was chosen so as to give a C: N of 20:1, which is the ratio found in their natural habitat.

Basal Modified Melin Norkran nutrient medium was prepared as described in Marx (1969) and the pH of medium was adjusted to 5.0. All inorganic chemicals and carbon sources were analytical grade. Media were autoclaved at 121 °C for 15 min. and allowed to cool at room temperature. The peptone was dissolved in basal medium and pH adjusted to 5.0 before filter sterilisation. This was then added to the already autoclaved medium, and 25 ml of each medium was poured into Petri dishes and inoculated with a 5 mm diameter inoculum plug from the edge of an actively growing colony. Static cultures were incubated at 30 °C in the dark. Harvesting was done at 10, 20 and 30 days. At each harvest all mycelium from each plate was collected and placed into pre-weighed aluminium boats. They were oven dried at 80 °C for 24 hours, then cooled in a desiccator before weighing. The pH of the medium was also determined.

Data was analysed statistically using Minitab 13. Analysis of variance was used to evaluate any differences in growth of isolates on the different carbon and nitrogen sources.

RESULTS

Scleroderma sinnamariense

Scleroderma made significant growth only on glucose as a carbon source in both ammonium-N (8.5 mg) and peptone-N (17 mg) (Figure 1a, b). Growth (17 mg) on glucose was significantly greater ($P<0.001$) when peptone was the N source (Figure 1b).

When ammonium was the N source there appeared to be minimal increase (2.8 mg) in biomass following growth in cellulose + starter glucose, and after 20 days there was some growth (3.6 mg) on sucrose (Figure 1a).

The fungus also showed minimal growth (2.6 mg) on cellulose + starter glucose with peptone as the N source, but the increase in biomass (2.6 mg) after 20 days when sucrose was C source was much less pronounced (Figure 1b).

Growth of this fungus on cellulose + starter glucose in both ammonium-N (2.5 mg) and peptone-N (3.5 mg) was better than on cellulose in absence of glucose for both N sources.

Pisolithus tinctorius

The growth curve for *Pisolithus* on ammonium-N (Figure 2a) with glucose as C source was different from that of *Scleroderma* in that there was a pronounced lag period. Once again growth was greatest (15 mg) on glucose, with some growth on sucrose (5 mg). There was also minimal biomass increase (2.6 mg) when cellulose + starter glucose were used, but no growth in the other C sources.

In contrasts to *Scleroderma*, growth of *Pisolithus* on all the carbon sources with peptone-N (Figure 2b) was much poorer than that on ammonium. There was a small biomass increase (4.5 mg) with glucose and a transitory weight gain on sucrose; an increase in biomass of 4.1 mg after 20 days of growth was followed by a decrease in biomass to 2.5 mg by day 30.

This fungus showed adaptive growth on cellulose + starter glucose in presence of ammonium-N (2.5 mg) and peptone-N (3.0 mg). Growth of *Pisolithus* in cellulose in the absence of glucose, with ammonium-N (2.0 mg) and peptone-N (2.2 mg) was slower.

pH changes

The final pH of solution following growth of *Scleroderma* and *Pisolithus* on different carbon sources is shown in Figures 3 and 4 respectively.

Utilisation of glucose, sucrose and starch by *Scleroderma* in ammonium-N led to a decrease in pH of the medium from 5.0 to 3.2, 3.8 and 4.8 respectively. *Pisolithus* also showed a similar pattern in which growth on glucose, sucrose and starch, in ammonium-N led to a decrease in pH of the medium from 5.0 to 3.4, 3.5 and 4.8 respectively. The decrease in pH was most significant for both isolates when carbon source was sucrose or glucose. Growth on cellulose by both *Scleroderma* and *Pisolithus* in ammonium-N led to an increase in pH of medium from 5.0 to 5.2 (Figures 3 and 4). Incubation of both fungi, on all carbon sources except glucose, in presence of peptone-N resulted in an increase in pH (Figures 3 and 4).

DISCUSSION

The utilisation of sucrose by *Scleroderma* only in ammonium-N might have been facilitated by changes in pH which would have occurred following ammonium uptake. Hampp and Schaeffer (1994) observed that sucrose hydrolysis was significant at pH below 4.0. In the current study, the media were not buffered and uptake of ammonium would have caused acidification of the medium containing glucose, sucrose and starch. The drop in pH may have favoured hydrolysis of the sucrose to its constituent glucose and fructose which might have been used by the fungus for growth. The *Pisolithus* isolate used in this study showed an increase in biomass following incubation on sucrose. This may also be as a result of the fall in pH. Salzer and Hager (1991) demonstrated that some EM fungi such as *Amanita muscaria* and *Hebeloma crustiliniforme* could not use sucrose directly but glucose and fructose were readily consumed. Although sucrose is a dominant plant sugar (Smith and Read, 2008), some researchers such as Taber and Taber (1987) and Salzer and Hager (1991) had attributed the inability of *P. tinctorius* to use sucrose to the absence of wall bound invertase that would enable them to hydrolyse this disaccharide to glucose and fructose.

Lamb (1974) observed growth of *Pisolithus tinctorius* and *Suillus bovinus* on sucrose, caused by introduction of 'starter' glucose. He called this adaptive growth. He also observed that prolonged incubation time had a positive effect on utilisation of the different carbon sources. In the current study, 'starter' glucose caused adaptive growth of both *Scleroderma* and *Pisolithus* in cellulose in the presence of ammonium and peptone-N. This adaptive growth observed was significant in the case of *Pisolithus* on peptone-N.

Table 1: Weight of carbon and nitrogen sources added to basal MMN nutrient solution for growth evaluation of Scleroderma sinnamariense.

Carbon sources	Ammonium-N		Peptone-N	
	Weight (g) of C source	Weight (g) of ammonium sulphate	Weight of C source	Weight (g) of peptone
Glucose	3.004	0.284	2.100	0.380
Sucrose	2.862	0.284	2.005	0.380
Soluble starch	2	0.284	2	0.380
Cellulose	2	0.284	2	0.380
Cellulose + 'starter' glucose	1.90 + 0.1	0.284	1.90 + 0.1	0.380

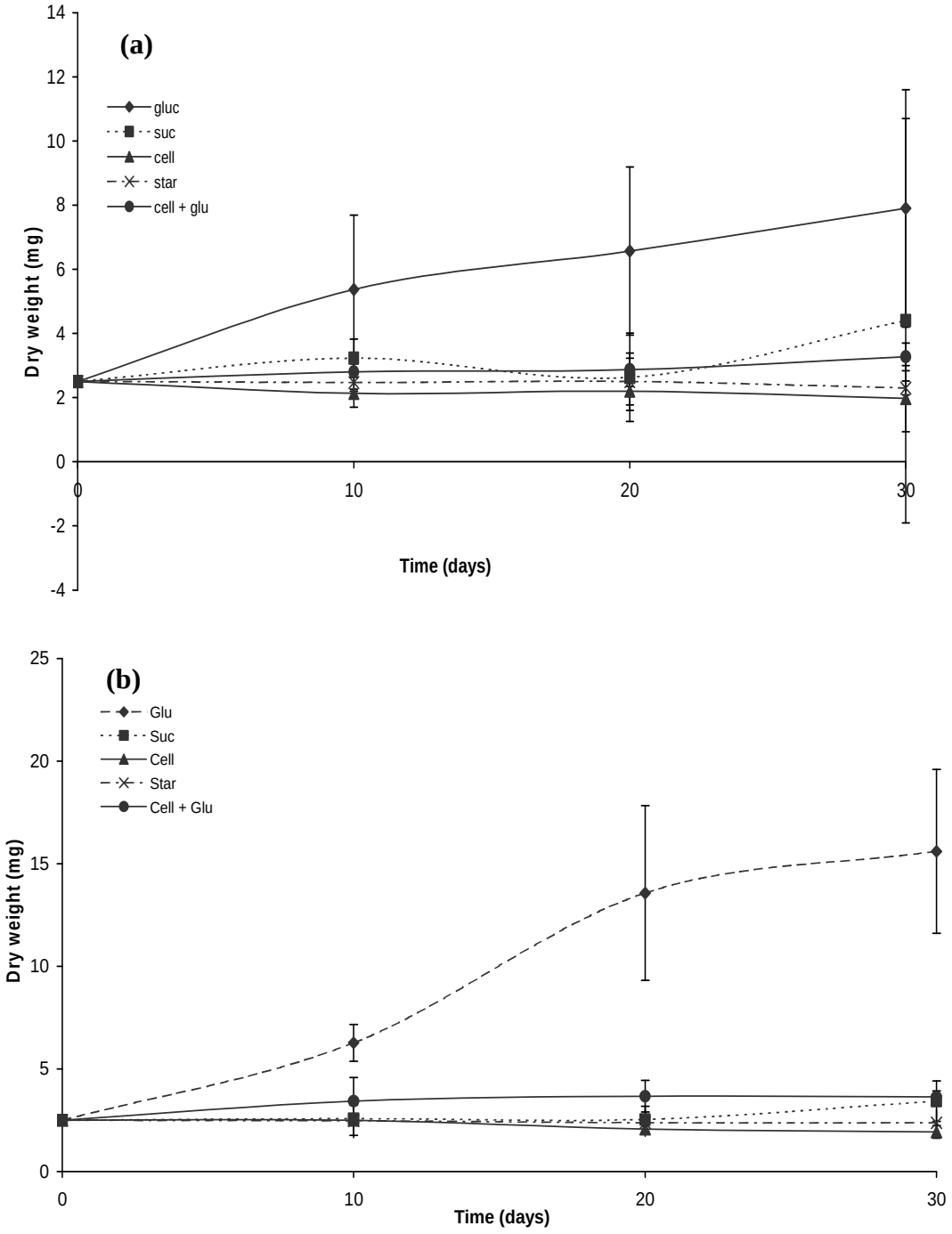
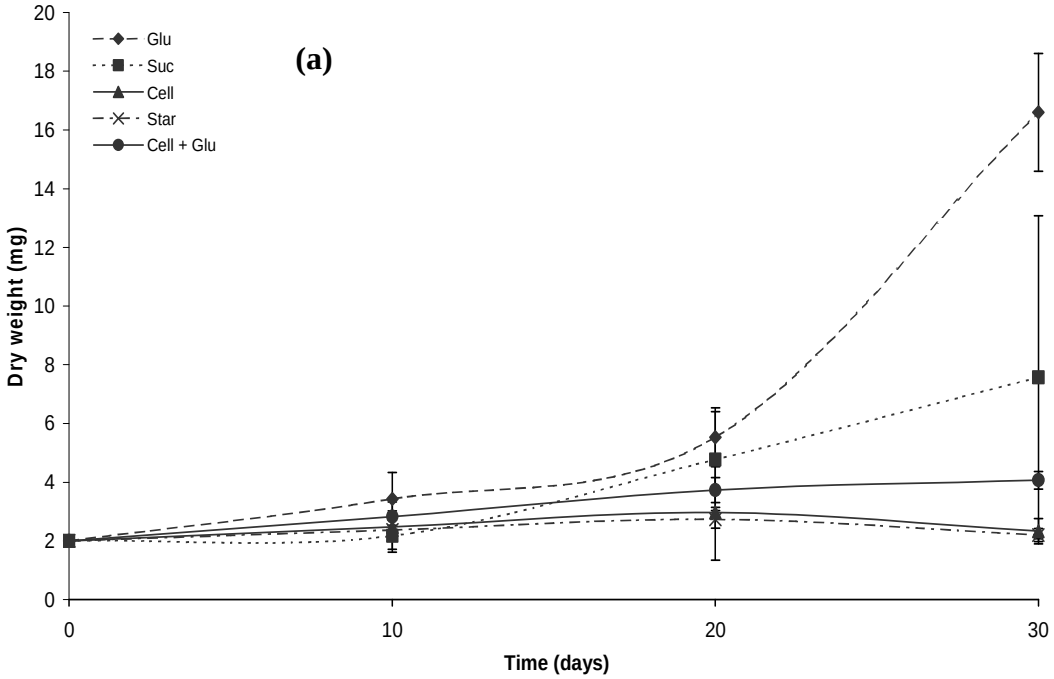


Figure 1: Dry weight yields of *Scleroderma* following growth on MMN nutrient medium containing different carbon sources with ammonium-N (**a**) and peptone-N (**b**). Each point is a mean of three replicates. Vertical bars represent 95% confidence interval.



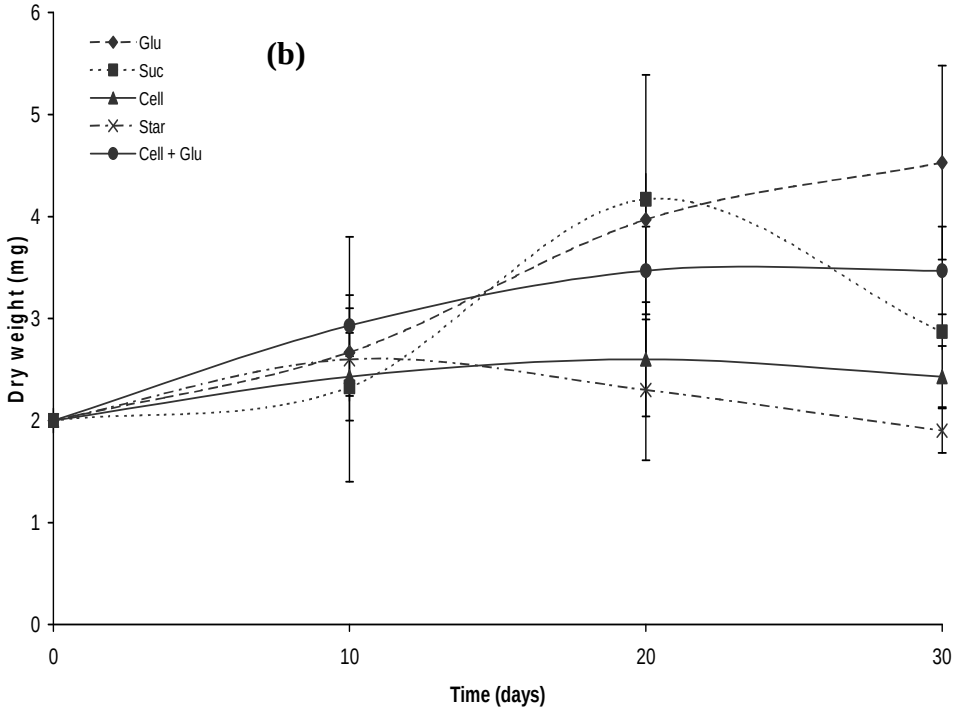


Figure 2: Dry weight yields of *Pisolithus* following growth on MMN nutrient medium containing different carbon sources with ammonium-N (**a**) and peptone-N (**b**). Each point is a mean of three replicates. Vertical bars represent 95% confidence interval.

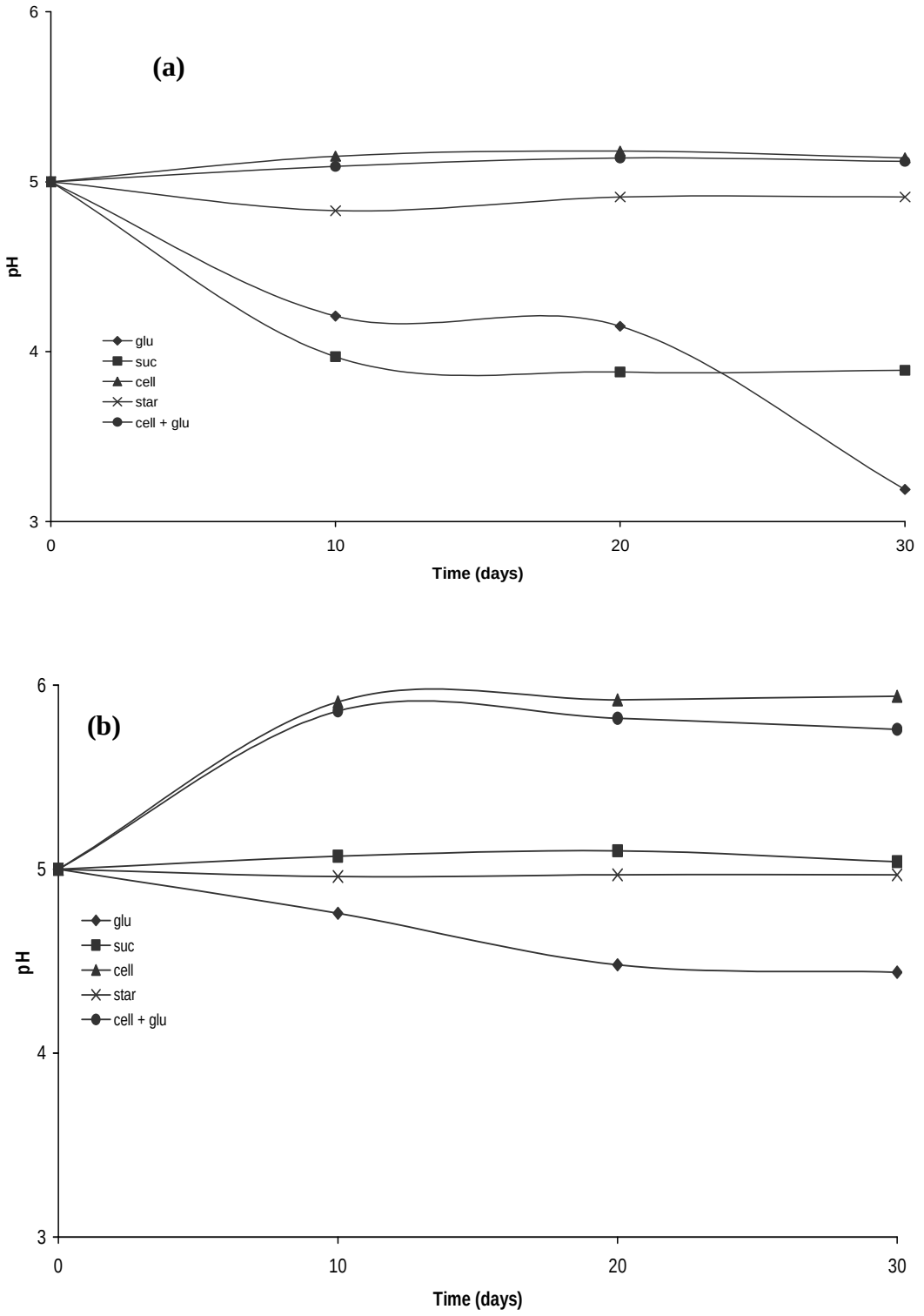


Figure 3: pH changes following growth of *Scleroderma* on different C sources in presence of ammonium-N (**a**) and peptone-N (**b**). Each point is a mean of three replicates.

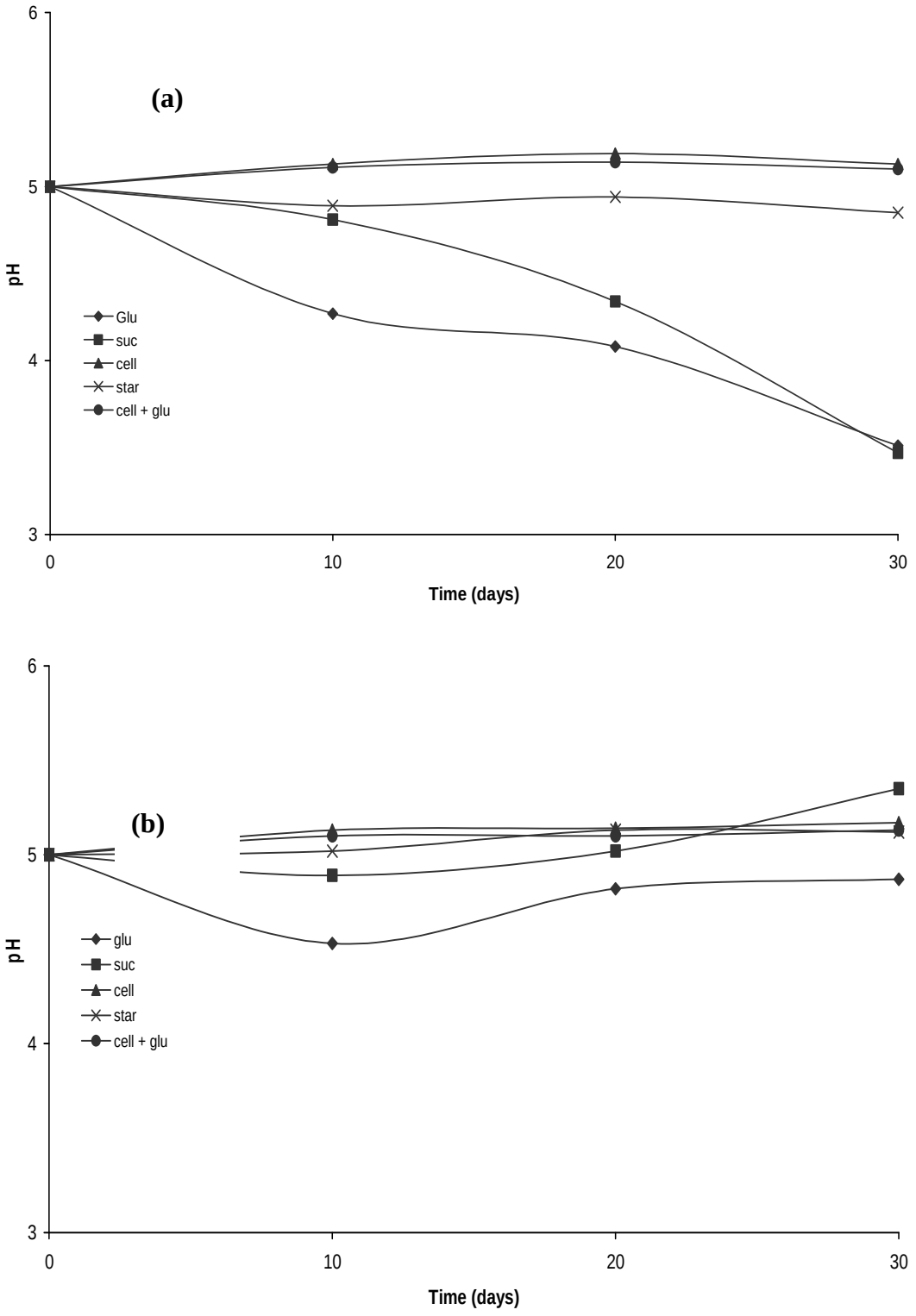


Figure 4: pH changes following growth of *Pisolithus* on different C sources in presence of ammonium-N (**a**) and peptone N (**b**). Each point is a mean of three replicates.

The increase in biomass observed following growth on carbon sources like starch and cellulose may have been as a result of the presence of small quantities of impurities in the growth medium. The thiamine-HCl contained carbon which might have acted as a starter. Peptone also contained carbon which may be used by the fungi. There was the possibility that a carry over of carbon from the inoculum plug may have occurred and served as a starter. Another possibility was that a partial hydrolysis of complex carbon sources may have occurred during autoclaving. Although the carbon sources assayed in the current study may be available in natural systems, it is probable that some fungi, in the absence of their host, may not readily utilize them.

This study demonstrated that utilisation of carbon sources was partially dependent on nitrogen source of the medium. It also showed that comparing relative utilisation percentages from different experiments may be misleading especially if the two experiments were not carried out on the same media containing the same N sources. There is a possibility that the limited growth of *Scleroderma* on sucrose and cellulose could restrict the fungi to the root of the host plant due to unavailability of nutrients.

The fact that most carbon utilisation studies on ectomycorrhiza fungi used basal media different from the medium used in the current study makes comparison difficult. Mikola (1948) and Keller (1952) found that *Cenococcum geophilum*, an ectomycorrhizal fungus, could use mannose, trehalose, cellobiose and alpha dextrin effectively as glucose, whereas growth on starch, cellulose, sorbitol, galactose, inulin and delta dextrin was poor. Mamoun and Olivier (1991) reported the ability of mannose, sucrose and trehalose to substantially support growth of *Tuber melanosporum*, *Cenococcum geophilum*, *Rhizopogon roseolus* and *Suillus bovinus*.

Hutchinson (1990) evaluated the ability of ninety-six species of ectomycorrhizal fungi from thirty genera to grow on MMN agar where the carbon (glucose) supply was replaced by cellulose, lignin, pectin, lipid, amylose and gelatin. He found that ectomycorrhizal fungi did not degrade cellulose, lignin or pectin. However some species of *Amanita* and *Cortinarius* produced lipases while other species of *Piloderma*, *Thelephora* and *Lactarius* produced gelatinases.

The current study is not conclusive of the ability of the EM fungus endophyte associated with *Gnetum* sp. to use carbon sources other than glucose. It is imperative to use a wider variety of carbon sources. If results from such *in vitro* studies have to be related to the function of the fungus *in vivo*, then the ability to degrade natural substrates like litter, twigs and leaves need to be evaluated.

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REFERENCES

Abuzinadah RA, Read DJ. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants I.: Utilisation of peptides and proteins by ectomycorrhizal fungi. *New Phytol.*, **103**: 481-493.

Bechem EE. 2004. Mycorrhiza status of *Gnetum* spp. in Cameroon: evaluating diversity with a view to ameliorating domestication efforts. PhD thesis, University of Aberdeen, Scotland, UK.

Bechem EE. 2011. Growth and *in vitro* phosphate solubilising ability of *Scleroderma sinnamariense*: A tropical mycorrhiza fungus isolated from *Gnetum africanum* ectomycorrhiza root tips. *J. Yeast and Fung. Res.*, **2**(9): 132–142.

Bechem EE, Alexander IJ. 2011. Phosphorus nutrition of ectomycorrhizal *Gnetum africanum* plantlets from Cameroon. *Plant Soil*, **353**: 379-393.

Cochrane VW. 1958. *Physiology of Fungi*. Whiley: New York.

Hampp R, Schaeffer C. 1994. Mycorrhiza-carbohydrate and energy metabolism In *Mycorrhiza-Structure, Function, Molecular Biology and Biotechnology*, Varma A, Hock B (eds). Springer-Verlag: Germany.

Hansen CA, Allison SD, Bradford MA, Wallenstein MD, Treseder KK. 2008. Fungal taxa target different carbon sources in forest soil. *Ecosyst.*, **11**: 1157-1167.

Högberg P, Högberg MN. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half of the dissolved organic carbon in a forest soil. *New Phytol.*, **154**: 791-795.

Hutchinson LJ. 1990. Studies on the systematics of ectomycorrhizal fungi in axenic culture.II. The enzymatic degradation of selected carbon and nitrogen compounds. *Can. J. Bot*., **68**: 1522-1530.

Johnson D, Leake JR, Ostle N, Ineson P, Read DJ. 2002. In situ $13CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytol.*, **153**: 327-334.

Keller HG. 1952. Untersuchungenber das Wachstum von *Cenococcum graniforme* (Sow.) Ferd. Winge auf verschiedenen Kohlenstoffquellen. Ein Beitrag zur Kenntnis der Physiologie der mykorrhizabildenen Pilze.- Thesis. Juris-Verlag, Zurich.

Lamb RJ. 1974. Effect of D-glucose on utilization of single carbon sources by ectomycorrhizal fungi. *Trans. Br. Mycol. Soc*., **63**(2): 295-306.

Mamoun M, Olivier JM. 1991. Effect of carbon and nitrogen sources on *in vitro* growth of *Tuber melanosporum* Vitt. Application to mycelia biomass production. *Agronomie,* **11**: 521-527.

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

Mikola, P. 1948. On the physiology and ecology of Cenococcum graniforme especially as mycorrhizal fungus of birch.-Communicationes. *Inst. Forest. Fenn*., **36**(3): 1-104.

Palmer JG, Hacskaylo E. 1970. Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. *Physol. Plant*, **23**: 1187-1197.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance. *New Phytol.*, **157**: 475–492.

Salzer P, Hager A. 1991. Sucrose utilisation of the ectomycorrhizal fungi *Amanita* muscaria and *Hebeloma crustuliniforme* depends on the cell wall-bound invertase activity of their host *Picea abies*. *Bot. Acta*, **104**: 439-445.

Simard SW, Jones MD, Durall DM. 2002. Carbon and nutrient fluxes within and between mycorrhizal plants. In *Mycorrhizal Ecology*, van der Heijden, Sanders I (eds). Springer-Verlag: Heidelberg; 33-61.

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

Tabe WA, Taber RA. 1987. Carbon nutrition and respiration of *Pisolithus tinctorius*. *Trans. Br. Mycol. Soc*., **89**(1): 13-26.

Talbot JM, Allison SD, Treseder KK. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil carbon dynamics in ecosystems under global change. *Functional Ecol.*, **22**: 955-963.

Tu C, Booker FL, Watson DM, Chen X, Rufty TW, Shi W, Hu SJ. 2006. Mycorrhizal mediation of plant N acquisition and residue decomposition: impact of mineral N inputs. *Glob. Change Biol.*, **12**: 793–803.