Growth characteristics, nitrogen uptake and enzyme activities of the nitrate-utilising ectomycorrhizal *Scleroderma verrucosum*

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The growth characteristics and uptake capacities for NO_3^- and NH_4^+, as well as activities of nitrogen assimilating-enzymes have been determined in *Scleroderma verrucosum*. Biomass, grown on agar or liquid MMN media, was higher on nitrate. When *S. verrucosum* was grown in the presence of equal amounts of ammonium and nitrate, uptake rate for NH_4^+ was considerably higher than that of NO_3^-. In addition, it was found that a limitation in ammonium concentration was a prerequisite for nitrate uptake. Nitrate reductase was stimulated when the fungus was transferred from ammonium to nitrate containing media, indicating that the enzyme was inducible by nitrate and repressed by ammonium. Glutamine synthetase and NAD-glutamate synthase activities were clearly detected in *S. verrucosum*, while the NADP-glutamate dehydrogenase was almost undetectable. This is consistent with the view that ammonium assimilation occurs through the GS/GOGAT cycle in *S. verrucosum*.

Nitrogen availability is frequently a major factor limiting forest growth and the contribution of ectomycorrhizal fungi to the nitrogen nutrition of their host plants has been demonstrated (Bowen & Smith, 1981; Finlay, Frostegard & Sonnerfeldt, 1992; Thomson *et al*., 1994; Généré, 1995). In ecosystems such as boreal forests, ammonium is a major inorganic nitrogen source and nitrate is usually available at low concentrations (Adam & Attiwill, 1982). In calcareous or amended soils, however, nitrate may become an increasingly important nitrogen source and the concentration of nitrate could exceed ammonium concentration by several fold (Clément, Garbaye & Le Tacon, 1977; Marschner, Häussling & George, 1991). The ability of the external mycelium to assimilate a wide range of inorganic and organic nitrogen compounds has been reported (Melin & Nilson, 1952, 1953; Abuzinadah & Read, 1986, 1988; Finlay *et al*., 1988, 1989). In pure culture, most ectomycorrhizal fungi grow better on ammonium than on nitrate (Lundeberg, 1970; Abuzinadah & Read, 1988; Finlay, Frostegard & Sonnerfeldt, 1992). Several ectomycorrhizal fungi, however, such as *Laccaria laccata* (Ahmad *et al*., 1990), *Pisolithus tinctorius* (France & Reid, 1984; Plassard *et al*., 1991) and *Hebeloma cylindrosporum* (Scheromm, Plassard & Salsac, 1990*a*, *b*) can easily take up and assimilate nitrate. Indeed, Sarjala (1990) reported that nitrate-reducing capacity differs among mycorrhizal species and differences between species and strains depend on the concentration of substrate available to the fungus.

It is generally accepted that ectomycorrhizal fungi assimilate NO_3^- through the involvement of nitrate reductase (EC 1.6.6.3) and nitrite reductase (EC 1.6.6.4) enzymes, while NH_4^+ produced by both enzymes or assimilated from the external mycelium will be metabolized into glutamate and glutamine by the action of NADP-dependent glutamate dehydrogenase (NADP-GDH, EC 1.4.1.4) and glutamine synthetase (GS, EC 6.3.1.2)/glutamate synthase (EC 1.4.1.14) (GS/GOGAT) cycle (Botton & Chalot, 1995). The physiology of nitrogen assimilation in ectomycorrhizal fungi is, however, still poorly understood. The absence of NADP-GDH in the ectomycorrhizal basidiomycetes *P. tinctorius* and *Paxillus involutus*, as demonstrated by enzyme activity measurement and Western-blotting, suggests that GS/GOGAT is the primary pathway for ammonium assimilation (Kershaw & Stewart, 1992; Chalot *et al*., 1994). Results based on ^{15}N-labelling of the ectomycorrhizal ascomycete *Cenococcum geophilum* suggest that its glutamine synthetase is highly active during the exponential phase of growth (Martin *et al*., 1988), whereas at the stationary phase, ammonium is assimilated essentially via the NADP-GDH pathway (Genetet, Martin & Stewart, 1984). The operation of the GDH pathway and the GS/GOGAT cycle were reported to be regulated by nutrient supply and growth conditions. NADP-GDH is responsible for ammonium assimilation in *Neurospora crassa* when ammonium is added in excess to the culture medium (Hernandez *et al*., 1983), whereas GS/GOGAT mainly operate in N-limited cultures (Lara *et al*., 1982).

The present study deals with the growth characteristics of the ectomycorrhizal basidiomycete *Scleroderma verrucosum*, an aggressive coloniser of *Eucalyptus globulus* plantations on ex-pasture sites in Australia, cultivated in the presence of nitrate or ammonium as the sole nitrogen source. The uptake of both ions used either individually or in combination and the

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enzyme activities related to ammonium and nitrate assimilation have been investigated.

MATERIALS AND METHODS

Fungal material. *Scleroderma verrucosum* (Bull.) Pers. (isolate MH13), originally isolated from under *Eucalyptus globulus*, was obtained from the Murdoch University mycorrhizal fungal collection, Perth, Australia. The isolate was maintained in Petri dishes on Modified Melin-Norkrans agar medium (MMN) at 24 °C.

Culture condition and biomass measurements. Fungal material was first transferred from the edges of MMN cultures to fresh liquid or agar MMN and grown axenically as described below. The MMN medium contained (mg l^{-1}): $MgSO_4 . 7H_2O$ (150), KCl (275), $CaCl_2 . 2H_2O$ (66), $(NH_4)_2SO_4$ (1250), $NaH_2PO_4 . 2H_2O$ (780), thiamine hydrochloride (1), biotin (0·4 µg), glucose (5 g l^{-1}) and micronutrients. When needed, $(NH_4)_2SO_4$ was replaced by $NaNO_3$ (1608 mg l^{-1}). For growth experiments on agar medium, mycelium was cut from the edge of 3 wk old colony, placed on a synthetic agar medium covered with cellophane and maintained in the dark at 24°. Growth on concentrations ranging from 0·9 to 36 mM of NH_4^+ or NO_3^- was measured after 4 wk and expressed as mg F.W. For experiments in non-stirred liquid medium, the inoculum was grown on the surface of 100 ml MMN media in 250 ml Erlenmeyer flasks, at pH 5·6 and maintained at 24°, shaken gently for 20 s at least once every 3 d and then daily to ensure oxygenation during the exponential period of growth.

Uptake experiments were done by transferring 2 wk old mycelia grown on liquid MMN containing 2 mM of NH_4^+ or NO_3^-, after a 24 h period of starvation. The uptake rate was determined by measuring the depletion of NH_4^+ or NO_3^- from the medium. The optimum pH for uptake rate was measured 3 d after transfer. The effect of NH_4^+ and/or NO_3^- concentration on ion uptake was investigated by varying the proportion of NH_4^+ in the incubation medium, while the NO_3^- concentration was kept at 2 mM. Uptake was measured every 24 h for 3 d.

Ammonium and nitrate analysis. The concentration of ammonium and nitrate in the culture media was measured by using a standard capillary electrophoresis method. Ammonium determination was carried out on a fused capillary (60 cm long and 52·5 cm effective length separation to sample loading, 75 µm internal diam., Supelco, Supelco Park, Bellefonte, U.S.A.) with UV-indirect detection at 185 nm (Quanta 4000, Waters Millipore), using the following buffer: tropolone (5 mM), 18 crown-16 ether (2 mM) and UV-cat 2 (3 mM). The voltage applied was 20 kV using positive polarity power supply which gave a current inside the capillary of 5·4 µA at room temperature. Separation of nitrate was performed with similar capillary conditions, except that the voltage was applied with a negative polarity and an internal current of 18·2 µA. The detection was carried out at 254 nm. Nitrate determination was achieved using sodium chromate (5 mM) and sulphuric acid buffer (0·68 mM) supplemented with 2·5% OFM (osmotic flow modifier; a surfactant for capillary electrophoresis (Waters)) (v/v), at pH 8. Identification and quantification of the compounds were performed with standard mixtures and co-injections.

Enzyme assays. Enzyme activities were measured during the growth period or after transfer of 2 wk old fungal mycelia grown on ammonium into a medium containing ammonium or nitrate. Mycelia were harvested periodically and used directly for enzyme determinations or frozen in liquid nitrogen, freeze dried and then stored at −80°.

The mycelium (50–200 mg F.W.) was homogenized using mortar and pestle at 4° with 2 ml extraction buffer containing 1 mM ethylenediaminetetraacetic acid (sodium salt), 14 mM 2-mercaptoethanol, 1 mM dithiothreitol, 5 mM $MgSO_4$, 10 mM Na-glutamate, 10% (v/v) glycerol and 2% soluble polyvinyl-pyrrolidone (mol. wt 40000) in 100 mM potassium phosphate, pH 7·5. After centrifugation at 14000 ***g*** for 15 min, the supernatant was collected and used for enzyme assays or protein analysis. Sodium glutamate was removed from the extracting buffer when the GOGAT enzyme was analysed.

Nitrate reductase was measured *in vivo* using a modification of the method described by Plassard, Mousain & Salsac (1984). Each colony of *Scleroderma verrucosum* was transferred into a test tube containing 5 ml of 50 mM phosphate buffer, pH 7·5, containing 100 mM potassium nitrate and 1% isopropanol (v/v). After 60 min at 30°, the reaction was stopped by incubating the tubes in boiling water for 2 min, then the solution was filtered. The nitrite content was measured in a mixture containing 1 ml of filtrate medium with 500 µl of 1% (w/v) sulphanilamide and 500 µl of 0·02% (w/v) N-(1-naphthyl)-ethylene diamine dihydrochloride in 3 M HCl. The absorbance was measured at 540 nm (Shimadzu UV-160) after 20 min of incubation in the dark. Fungal colonies were omitted in the control samples. The *in vivo* activity of nitrate reductase was expressed as µmol nitrite released in the incubation mixture (h^{-1} g^{-1} F.W.).

The activity of nitrate reductase was measured *in vitro* using a modification of the method described by Essgaouri & Botton (1990). The mycelium was homogenized in 1 ml of extraction buffer containing 100 mM potassium phosphate buffer, pH 7·5, 10 µM flavin adenine dinucleotide and centrifuged at 14000 ***g*** for 15 min. The reaction was started by adding 100 or 200 µl of the supernatant to the reaction mixture containing 50 mM potassium phosphate buffer, pH 7·5, 0·24 mM NADPH, and 10 mM potassium nitrate in a final volume of 1 ml. After 30 min incubation at 30°, the reaction was stopped by adding 100 µl 1 M $ZnSO_4$ and the mixture was centrifuged at 14000 ***g*** for 10 min. Nitrite was estimated in 1 ml of supernatant as described for the *in vivo* assay. Three independent measurements were performed for each experiment.

The activities of glutamine synthetase and glutamate dehydrogenases were determined according to Brun *et al.* (1992). Glutamate synthase activity was estimated according to the method described by Matoh, Ida & Takahashi (1980). Aspartate aminotransferase and alanine aminotransferase were measured according to Khalid *et al.* (1988).

RESULTS

Growth characteristics of the fungus

Figure 1 shows the effect of various concentrations of NH_4^+ and NO_3^- on growth of *S. verrucosum*. The optimum concentrations for growth were 2·25 and 4·5 mM for NH_4^+ and NO_3^-, respectively. At higher concentrations, growth on nitrate levelled off whereas that on ammonium decreased. For all concentrations tested, growth on NO_3^- was 2- to 3-fold higher than that on NH_4^+. When NH_4^+ was replaced by 5 mM amino acids, growth was similar in glutamate supplemented media but lower in the presence of glutamine and especially in the presence of aspartate and alanine (Table 1).

In liquid culture similar results were observed (Fig. 2). The fungal biomass on nitrate was 35% higher than that on ammonium at the end of the exponential growth period (6 wk). The pH of the culture medium was initially adjusted to 5·6 and gradually increased to pH 6·9 on nitrate, whereas it decreased to pH 3·3 on ammonium (Fig. 3).

Ammonium and nitrate uptake

There was no clear-cut effect of the pH on ammonium consumption whereas nitrate consumption was optimum at pH 5·1 when measured in non-buffered medium (Fig. 4). Ammonium consumption was, however, faster than nitrate consumption. Indeed, after 3 d of incubation, 68% of ammonium but only 45% of nitrate was taken up.

When the mycelium was transferred into a medium containing NH_4^+ and NO_3^- in approximately the same proportion (1·93 mM and 2·06 mM), NH_4^+ uptake reached a value of 16·9 μmol h^{-1} g^{-1} D.W. after 2 d of incubation and then decreased by 2·5 times after 3 d (Fig. 5). NO_3^- uptake was almost undetectable during the 3 d of incubation (Fig. 5). When NH_4^+ concentration was lowered to 1/5 or 1/20 of the NO_3^- concentration, NH_4^+ uptake increased during the first 2 d and then decreased (Fig. 5). Uptake values were, however, 3- to 5-fold lower when compared with the high NH_4^+ concentration. Interestingly, NO_3^- uptake was still undetectable during the first day of incubation, but greatly increased to a value of 2·7 μmol h^{-1} g^{-1} D.W. when ammonium was lowered to 1/5 of the initial concentration during the second day and became higher than NH_4^+ uptake during the third day. Results were even more obvious when NH_4^+ was reduced to 1/20 of the initial concentration since NO_3^- uptake became higher than NH_4^+ uptake after 2 d of incubation. The pH of the incubation medium (initially 5·1) decreased to 3·1 and 4·0 for the highest and 1/5 of the initial concentration while at the lowest ammonium concentration, the pH was increased to 5·8. Additionally, it appeared that when NH_4^+ and NO_3^- were given at equal concentrations, the *in vitro* NR activity was almost undetectable (32 μmol NO_3^- h^{-1} g^{-1} F.W.) whereas it increased by 10- and 15-fold when NH_4^+ was lowered to 1/20 and 1/5 of the NO_3^- concentration, respectively (not shown).

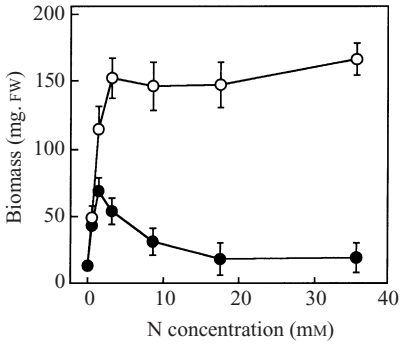
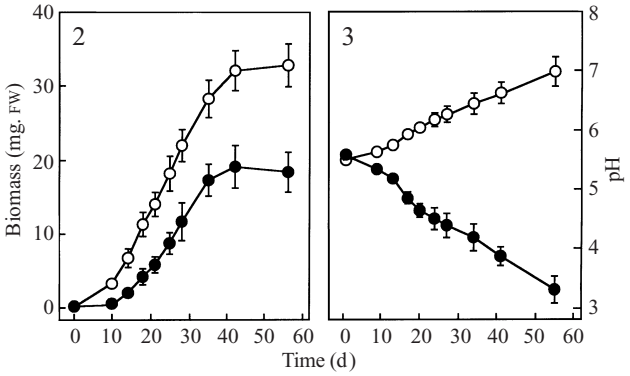


Fig. 1. Growth of *Scleroderma verrucosum*, as a function of increasing concentrations of ammonium (●) and nitrate (○), grown on MMN agar media and harvested after 4 wk. Values are means (±S.E.) of six cultures.

Table 1. Growth of 3-wk-old colonies of *Scleroderma verrucosum* on agar media supplemented with 5 mM of inorganic or organic nitrogen sources. Values are means (±S.E.) of 6 cultures

N sources	mg F.W.
NH_4^+	65·1 ± 10·5
NO_3^-	152·6 ± 14·6
NH_4NO_3	72·3 ± 11·1
Ala	28·2 ± 10·2
Asp	42·2 ± 11·4
Glu	69·8 ± 12·7
Gln	57·6 ± 8·3



Figs 2, 3. Influence of ammonium (●) and nitrate (○) on growth of *Scleroderma verrucosum* colonies (**Fig. 2**) and on pH evolution of the culture medium (**Fig. 3**). Values are means (±S.E.) of four replicates.

Regulation of enzyme activities

Table 2 shows the activities of nitrogen-assimilating enzymes in 3 wk old mycelia growing on ammonium or nitrate medium. The activities of the glutamine synthetase and the aminotransferases (aspartate aminotransferase, AAT, and alanine aminotransferase, AlAT) were higher on nitrate than on ammonium. By contrast, the activities of NAD-dependent glutamate dehydrogenase and NAD-dependent glutamate synthase were higher on ammonium. The activity of NADP-

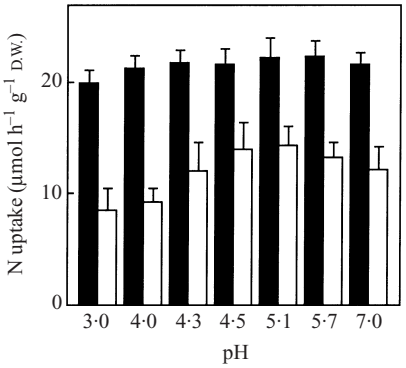


Fig. 4. Inorganic nitrogen uptake by *Scleroderma verrucosum* incubated for 3 d on ammonium (■) and nitrate (□) at different pH values. Mycelia were N-starved for 24 h before being transferred to nitrogen supplemented media. Values are means (±S.E.) of three replicates.

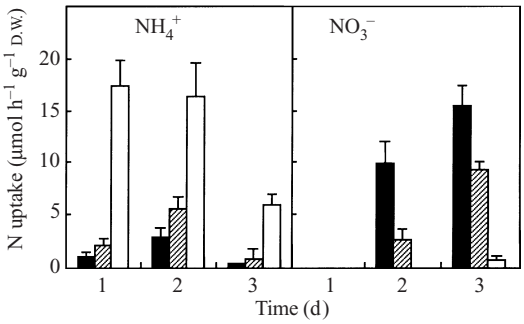
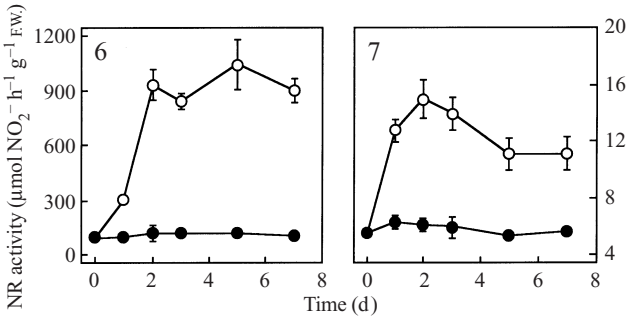


Fig. 5. Uptake of ammonium and nitrate by *Scleroderma verrucosum* incubated for 3 d on different concentrations of ammonium and nitrate. ■, [0·16]/[1·97]; ▨, [0·36]/[1·90] and □, [1·93]/[2·06]. Mycelia were N-starved for 24 h before being transferred on nitrogen supplemented media. Values presented are means (±S.E.) of three replicates.



Figs 6, 7. Nitrate reductase activities measured *in vitro* (**Fig. 6**) and *in vivo* (**Fig. 7**) in 2-wk-old cultures of *Scleroderma verrucosum*. Mycelia were N-starved for 24 h before being transferred to ammonium (●, control) and nitrate (○) supplemented media. Values are means (±S.E.) of three replicates.

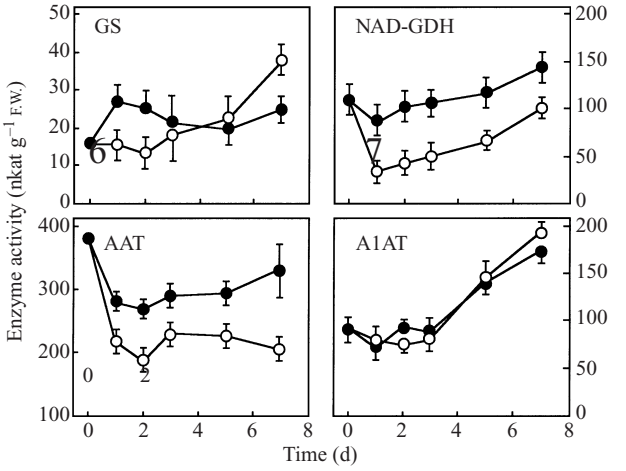


Fig. 8. Time courses of enzyme activities (GS, NAD-GDH, AAT and AlAT) in 2-wk-old cultures of *Scleroderma verrucosum* cultivated in the presence of ammonium (●) and nitrate (○). Mycelia were N-starved for 24 h before being transferred on nitrogen supplemented media. Values are means (±S.E.) of three replicates.

dependent glutamate dehydrogenase was almost undetectable both on ammonium and nitrate. The nitrate reductase activity was almost undetectable on NH_4^+ but very high on NO_3^-. Additionally, the *in vitro* nitrate reductase activity was 33 times greater than the *in vivo* nitrate reductase activity. Furthermore, kinetic studies performed during the growth period showed that glutamine synthetase activity was higher at the exponential period and was lower on ammonium than on nitrate while the aminotransferases increased until the end of the exponential growth period and then decreased afterwards (results not shown).

In order to determine the induction period for the nitrate reductase activity, mycelia were grown on NH_4^+ for 2 wk, starved for 24 h and transferred on nitrate or ammonium. Nitrate reductase activity as well as other NH_4^+ metabolizing enzyme activities were measured daily for 1 wk. The *in vitro* (Fig. 6) and *in vivo* (Fig. 7) assays for NR activities increased by 10- and 3-fold, respectively, after 2 d incubation on nitrate supplemented media. In the ammonium containing medium, NR activity remained almost undetectable (Figs 6, 7). The activities of NAD-GDH and AAT decreased during the first 2 d of incubation and then increased (Fig. 8). This initial decrease was, however, more pronounced on NO_3^- medium. AlAT showed no differences between NH_4^+ or NO_3^-, both increasing with time, especially after 3 d incubation. GS

Table 2. Enzyme activities of 3-wk-old *Scleroderma verrucosum* grown in liquid culture supplemented with ammonium or nitrate as sole nitrogen source. Enzyme activities are expressed in nkat (mg protein)$^{-1}$, except NR which is expressed as μmol NO_2^-.h^{-1} g^{-1} F.W. Values are means (±S.E.) of three replicates

	Nitrate reductase					GDH		GOGAT
	in vivo	*in vitro*	AAT	AlAT	GS	NADH	NADPH	NADH
NH_4^+	0·3±0·1	7·7±1·1	31·8±1·5	17·5±0·3	1·7±0·1	18·6±0·2	0·02±0·02	1·3±0·4
NO_3^-	25·8±3·8	844·1±35·1	40·2±0·9	30·1±1·6	4·5±0·6	11·9±0·4	n.d.	0·5±0·1

n.d. not detectable.

activity increased in the first day and then levelled off on ammonium. When transferred on NO_3^-, there was a significant increase in GS activity at the end of the incubation period.

DISCUSSION

Growth of *S. verrucosum* in non-buffered liquid culture or on solid agar media was higher on nitrate than on ammonium. This marked preference for NO_3^- has already been found with *Hebeloma cylindrosporum* (Scheromm, Plassard & Salsac, 1990*a*, *b*). According to those authors, however, other fungi such as *Cenococcum geophilum*, *Thelephora terrestris*, *Laccaria laccata*, *Hebeloma crustuliniforme* and *Piloderma croceum* grew better on ammonium. For *H. crustuliniforme*, the biomass production in non-buffered media was reduced due to the low pH (Littke, Bledsoe & Edmonds, 1984). *Laccaria proxima*, *Paxillus involutus* (Finlay, Frostegard & Sonnerfeldt, 1992), *Pisolithus tinctorius* and *Suillus granulatus* (France & Reid, 1984) grew equally well on nitrate or ammonium. The lower biomass yields of *S. verrucosum* cultivated on ammonium may be due to the fact that the pH of the culture media decreased to 3 at the end of the experiment. In addition, by increasing ammonium concentration, growth of *S. verrucosum* declined, while on nitrate, growth of the fungus was not affected.

When both N sources were provided at equal concentrations, ammonium was preferred as demonstrated by the high NH_4^+ and the low NO_3^- uptake rates during 3 d of incubation. When the NH_4^+ concentration was lowered, NO_3^- uptake increased but only after 2 d of incubation, indicating that NH_4^+ was a hindrance to the utilization of nitrate.

In general, the question of ammonium or nitrate preference by mycorrhizal fungi appears to be highly species specific (Bowen & Smith, 1981). The studies carried out on several ectomycorrhizal fungi showed that NH_4^+ uptake was faster than NO_3^- uptake. For example, NH_4^+ uptake by *H. crustuliniforme* was 310 μmol g^{-1} h^{-1} D.W. that is eight times higher than NO_3^- uptake (39 μmol g^{-1} h^{-1} D.W.) (Littke, Bledsoe & Edmonds, 1984). In contrast, for *C. geophilum*, NH_4^+ uptake was lower (19 μmol g^{-1} h^{-1} D.W.) than NO_3^- uptake (43 μmol g^{-1} h^{-1} D.W.) (Genetet, 1983). Most of these results were, however, obtained in specific culture conditions where a single form of nitrogen was provided to the fungus and little is known about the uptake rates of ectomycorrhizal fungi in the presence of combined N inorganic sources.

In higher plants such as *Betula verrucosa*, Ingestad (1976) showed that when combining NH_4^+ and NO_3^- in a 60:40 proportion, the net uptake of N-NO_3^- was 2-fold lower than that of N-NH_4^+. Moreover, Marschner, Häussling & George (1991) found that the net uptake of NO_3^- by non-mycorrhizal Norway spruce was very low until the concentration of NH_4^+ had declined below 100 μM, irrespective of the external NO_3^- concentration.

Similar observations were made by several authors in mycorrhizal associations where ammonium uptake was much higher than nitrate. Finlay *et al*. (1989) found that in beech ectomycorrhiza inoculated with *P. involutus*, the amount of nitrogen assimilated from nitrate was only 62% of that assimilated from ammonium (100%). Recently, Ek *et al*. (1994) confirmed that nitrogen uptake by *P. involutus* and transfer to spruce and birch ectomycorrhiza were significantly higher for NH_4-N than for NO_3-N. By feeding the fungal mycelium with ^{15}N-labelled in the form of $^{15}NH_4$-NO_3 or NH_4-$^{15}NO_3$, they found that ^{15}N-NH_4 was immediately assimilated into amino acids and then translocated by the fungal mycelium to the mycorrhizal roots. They suggested that N-NO_3^- was not directly assimilated in the mycelium but rather transferred to the mycorrhizal roots as nitrate.

Our results also demonstrated that NO_3^- uptake in *S. verrucosum* was strongly correlated with NR activity. When NH_4^+ ions were still present in the incubation medium, NO_3^- uptake was almost undetectable and NR activity remained negligable. The NR activity of *S. verrucosum* appeared to differ from that of *H. cylindrosporum* (Scheromm, Plassard & Salsac, 1990*b*), however, since thalli of the latter fungus grown on ammonium had a NR activity equivalent to that of thalli grown on nitrate. Also in this fungus, nitrate was not necessary for the expression of NR activity. In the ectomycorrhizal *S. verrucosum*, the NR activity appeared to be nitrate-inducible and was strongly repressed by ammonium. These results are in good agreement with those found in other fungi such as *N. crassa*, *Aspergillus nidulans* (Sorger & Davies, 1973; Dantzig *et al*., 1978; Garrett & Amy, 1978; Guerrero, Vega & Losada, 1981) and the ascomycete *Sphaerostilbe repens* (Essgaouri & Botton, 1990). Indeed, studies carried out on mutant strains of *N. crassa* lacking glutamine synthesis showed that it is not the ammonium itself but rather glutamine which was responsible for this repression (Premakumar, Sorger & Gooden, 1979).

Enzyme activity showed that glutamine synthetase was operative in *S. verrucosum*, while the NADP-glutamate dehydrogenase was almost undetectable. The lack of NADP-GDH activity is in good agreement with recent experiments which have shown that in four ectomycorrhizal fungi (species of *Elaphomyces*, *Amanita*, *Pisolithus* and *Gautieria*) glutamine was the major product accumulated following transfer of nitrogen-limited cultures to nitrogen containing media (Turnbull, Goodal & Stewart, 1996). In three of the fungi (species of *Amanita*, *Pisolithus* and *Gautieria*) glutamine synthesis was almost completely blocked by methionine sulphoximine and there was no incorporation of ^{15}N into glutamine. *Elaphomyces* displayed high incorporation of labelled ammonium even in the presence of MSX. Given the fact that GDH activity is substrate limited, however, as demonstrated in *Laccaria laccata* (Brun *et al*., 1992), the authors concluded that it seems unlikely that GDH would play a significant role in ammonium assimilation under field conditions. These results are in agreement with those from Rudawska *et al*. (1994) which suggested that the GS pathway for ammonium assimilation is potentially operative in ectomycorrhizal fungi isolated from *Pinus sylvestris* and which implied only a minor role for GDH.

Future work will address the presence of transport mechanisms and the regulation of nitrate reduction in *S. verrucosum*.

Financial support from the Indonesian Minister of Education and Culture as a grant fellowship to DPP is gratefully acknowledged.

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(*Accepted 4 October 1998*)