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Endogenous polyamine content and metabolism in the ectomycorrhizal fungus *Paxillus involutus*

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SUMMARY

Endogenous polyamine content of the ectomycorrhizal fungus *Paxillus involutus*, as well as the activity of its biosynthetic enzymes in relation to mycelia ageing were investigated in this work. Polyamines in free, PCA-soluble and insoluble conjugated forms, are present in *Paxillus involutus* mycelia in relatively high amounts and the ratio of putrescine to spermidine is age-dependent. Both arginine- and ornithine-decarboxylases are present, but putrescine biosynthesis proceeds mostly via ornithine decarboxylase and decreases with the age of mycelia. There was a large release of free polyamines from mycelia which showed age-dependent features. Clear polyamine uptake was observed in 2-wk-old mycelia and no competition between putrescine and cadaverine was detected. Putrescine uptake seems to reduce ornithine decarboxylase activity, but does not affect arginine decarboxylase.

Key words: arginine decarboxylase, ornithine decarboxylase, *Paxillus involutus*, polyamines, polyamine release, polyamine uptake.

INTRODUCTION

The predominant form of N in most temperate and boreal forest soils is organic N (Aguilera *et al.*, 1993) and it has been demonstrated that microorganisms are able to incorporate low molecular weight organic N compounds (e.g. amino acids) that result from organic matter breakdown, and directly assimilate the amino groups (Hadas *et al.*, 1992). Experiments performed by Finlay *et al.* (1988, 1989) showed that a large part of the N absorbed by mycorrhizal roots can be in organic form. Ectomycorrhizal mycelium represents a large part of the microbial mass in many forest soils and many of the metabolic interactions between the fungus and the host plant have still to be clarified. Ectomycorrhizal fungi (ECM) have the capacity to utilize organic N and the ECM *Paxillus involutus* has been shown actively to metabolize amino acids (Chalot *et al.*, 1994a,b) and to retain much of the assimilated N within its own tissues. Moreover, transfer of N compounds from protein to the associated host plants has been shown to vary according to the fungal species involved in the symbiosis (Abuzinadah & Read, 1989).

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Aliphatic polyamines are ubiquitous, low molecular weight, cationic molecules that play a role in a variety of physiological processes such as cell division, embryogenesis and response to various stresses (Bagni, 1989; Flores, 1991). The diamines putrescine and cadaverine were first identified in animal tissues subject to bacterial decay as result of a decarboxylation of ornithine and lysine respectively, and are therefore quite well represented in forest soils. They represent the most common diamines, spermidine and spermine being the most common polyamines, and they are degraded by diamine- and polyamine-oxidases respectively.

Detailed studies of short-distance transport of polyamines indicate the existence of specific transporters in higher plants (Antognoni *et al.*, 1993). Previous studies have also demonstrated that polyamines are translocated within the plant from roots to shoots and vice versa (Rabiti *et al.*, 1989; Caffaro *et al.*, 1993, Antognoni *et al.*, 1998).

Studies of free polyamine content have already been performed on *Paxillus involutus* by Zarb & Walters (1994a, 1995, 1996), to analyse the effects produced by inhibitors of polyamine biosynthesis and some toxic metals. Activities of enzymes involved in polyamine biosynthetic and oxidative pathways were also investigated.

The object of the present study was to increase knowledge of polyamine content of *Paxillus involutus*. In particular we intended to outline changes, both in free and conjugated forms, to the ageing of mycelia. Moreover, we made further determinations of the activities of two enzymes involved in putrescine biosynthesis: arginine decarboxylase (ADC) and ornithine decarboxylase (ODC). An additional aim was to demonstrate polyamine release and uptake capacity. In fact, since translocation of polyamines in pine seedlings has been demonstrated by Rabiti *et al*. (1989), and if polyamines are both absorbed and released by the mycelium of an ECM fungus, it is also possible that host roots in a symbiotic relationship can take up and translocate polyamines released by the fungal partner.

MATERIALS AND METHODS

Growth of the fungus

The isolate of *Paxillus involutus* Fr. was obtained from a fruitbody under a *Pinus sylvestris* L. stand on drained peatland in Alkkia, Finland and cultured in Petri dishes at room temperature on modified Melin–Norkrans medium (MMN).

Endogenous polyamine concentration and release

Discs of mycelium were cut from actively growing edges of colonies of fungus and placed in the middle of Petri dishes on MMN medium. The fungal colonies were grown for 4 wk and the position of the edge of the mycelial margin was marked weekly on the bottom of the dish. Discs were then cut for polyamine analysis from different areas of the mycelium after 1, 2, 3 and 4 wk of growth. To measure the release of polyamines, the agar under the mycelium was collected, care being taken to prevent removal of hyphae, and analysed for polyamines. Because MMN medium contains small amounts of free polyamines, the background amount of polyamines in MMN from Petri dishes without the fungus was subtracted from that of the agar taken from under the mycelium. Three replicate samples were used in every assay and treatment.

Paxillus involutus was also inoculated onto Petri dishes in liquid MMN medium from which malt extract, the source of polyamines, had been omitted. After 3 wk, polyamines were analysed from the liquid medium, which was first filtered in order to remove the mycelium. A slower growth rate of *Paxillus involutus* was observed on liquid media.

Polyamine loading

Polyamine uptake by *Paxillus involutus* was studied with mycelium grown on liquid MMN without malt extract by loading exogenous putrescine and cadaverine into the medium. Two week-old mycelia were incubated for 1, 4 and 8 h with fresh liquid MMN (without malt extract) containing 0, 0.1, 0.5 or 1 mM of putrescine. Cadaverine, which is not present in the endogenous pool, was supplied in different concentrations (0, 0.2 or 1.0 mM) with or without putrescine (0, 0.2 or 1.0 mM) for 1 to 4 h. The fungal mycelium was collected, washed twice with distilled water in order to remove any exogenous polyamines, weighed and frozen until analysed.

Polyamine analysis by HPLC

Samples were extracted in 5% $HClO_4$ and centrifuged at 37000 ***g*** for 15 min. The pellet was resuspended in the original volume of $HClO_4$. Duplicates of this suspension and of the supernatant were hydrolysed at 100°C for 16 h with 6N HCl in order to release polyamines from their conjugated forms.

Crude (for free polyamines) and hydrolysed (for soluble conjugated polyamines) supernatant and hydrolysed pellet (for insoluble conjugated polyamines) were dansylated and separated by HPLC using a modification of the procedure described by Smith & Davies (1985). Aliquots (200 µl) of $HClO_4$ extract were incubated overnight with 400 µl of fresh dansyl chloride (5 mg ml^{-1} of acetone) and 200 µl of saturated Na_2CO_3. The next day, 200 µl of proline (100 mg ml^{-1} H_2O) were added and samples were incubated for 30 min. Polyamines were then extracted into 400 µl of toluene, and 200 µl of the organic phase was evaporated at room temperature. Polyamine standards (purchased from Sigma Co., St Louis, MO, USA) were treated in the same way. Dansylated polyamines were dissolved in methanol and separated by HPLC (Merck Hitachi Model L-6200 Intelligent pump (Merck, Darmstadt, Germany), T-6300 column thermostat, D-2500 chromato-integrator), using a LiChroCART 125–4 LiChrospher 100 RP-18 5 µm column (Merck) and a methanol-water gradient. Polyamine concentrations were determined by fluorescence spectrophotometry (Merck Hitachi F-1050, Merck).

ADC and ODC activity assays

Arginine decarboxylase and ornithine decarboxylase activities were determined by a radiochemical method (Torrigiani *et al*., 1987).

Fresh mycelia were homogenized in an ice-cold mortar in 5 volumes of 100 mM Tris HCl buffer, optimum pH 8.5, with 50 µM of pyridoxal phosphate and 5 mM of dithiothreitol, and centrifuged at 26000 ***g*** for 30 min at 4°C. Aliquots (0.2 ml) of supernatant or resuspended pellet were incubated with 7.4 KBq of [U-^{14}C]-labelled arginine or [1-^{14}C]ornithine (Amersham International, Little Chalfont, Bucks,

UK) and different concentrations of unlabelled substrate (0–10 mM arginine, 0–10 mM ornithine). The presence of true ADC and ODC activities was confirmed by their determination in presence of a high concentration (10 mM) of exogenous unlabelled ornithine and arginine respectively, in order to inhibit any arginase or ornithine transcarbamylase activities. The reaction was carried out for 2 h at 37°C and stopped by the addition of 0.2 ml of 5% perchloric acid. The $^{14}CO_2$ released from the reaction mixture and captured by 200 µl of BTS (Beckman Tissue Solubilizer 450, Beckman Instruments, Fullerton, CA, USA) was then measured using a liquid scintillation analyser.

Protein concentration was determined following the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

RESULTS

Endogenous polyamine content

Free and conjugated polyamines were analysed in *Paxillus involutus* mycelium of different ages (1–4 wk). Free putrescine and spermidine were the major polyamines (Fig. 1a), whereas spermine was present only in traces. No cadaverine or 1,3-diaminopropane were found in the free or conjugated polyamine pools.

Soluble conjugated polyamines were present only in traces, whereas putrescine and spermidine constituted the insoluble conjugated pool (Fig. 1b). The total free polyamine content (400–650 µM) increased with the age of the mycelium, with putrescine the major polyamine in the young mycelium, and spermidine predominant in the oldest.

The total free and insoluble conjugated pattern showed the opposite trend with respect to growth of the mycelium. The oldest mycelia contained more free polyamines than the young, whereas the situation was reversed in the insoluble forms.

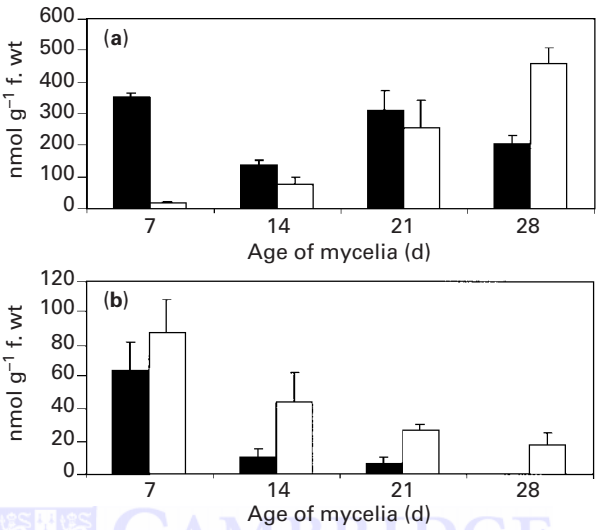

Fig. 1. Polyamine content in *Paxillus involutus* mycelia of different ages. (a) Free. (b) Insoluble conjugated. Closed squares, putrescine; open squares, spermidine.

Table 1. *Free polyamines in MMN agar medium under 1–4-wk-old* Paxillus involutus *mycelia*

Age (wk)	Putrescine (nmol g^{-1} f. wt)	Spermidine (nmol g^{-1} f. wt)
1	−19.62±2.91[a]	4.81±1.79
2	12.63±3.13	19.02±2.64
3	388.30±38.80	350.47±37.43
4	1964.67±412.42	2146.56±453.86

[a], the negative value indicates a putrescine uptake measured in a 1-wk-old mycelium.

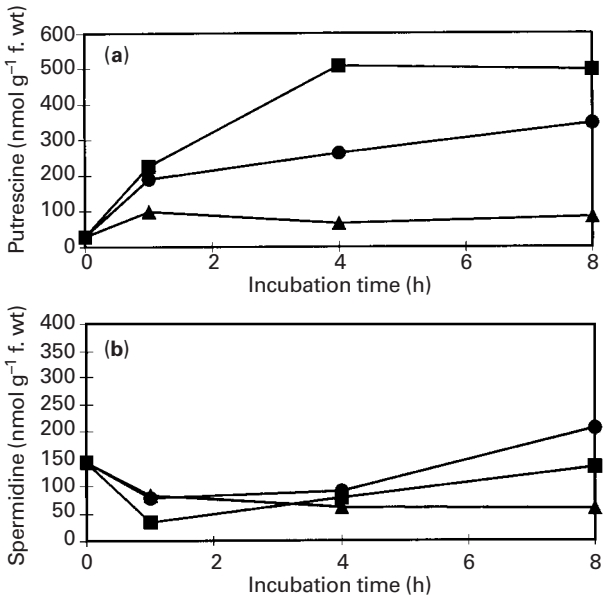

Fig. 2. Changes in (a) free putrescine and (b) spermidine content in 2-wk-old *Paxillus involutus* mycelia grown in liquid medium following incubation with different exogenous putrescine concentrations (closed triangles, 0.1 mM; closed circles, 0.5 mM; closed squares, 1 mM).

Polyamine release from Paxillus involutus *mycelium*

Polyamines from the agar collected from under 1–4 wk-old mycelia were analysed. No conjugated forms were detected: only free putrescine and spermidine were released (Table 1) and a small amount of free spermine was released from the oldest mycelium (10.15±1.91 nmol g^{-1} f. wt). The release rate increased with age of the mycelium. The same experiment was performed on mycelia grown for 3 wk in liquid media: again, no conjugated polyamines were found, but the amount of free polyamines detected was dramatically smaller than in the mycelium grown on solid medium (1.02±0.15 nmol g^{-1} f. wt of putrescine and 0.09±0.03 nmol g^{-1}

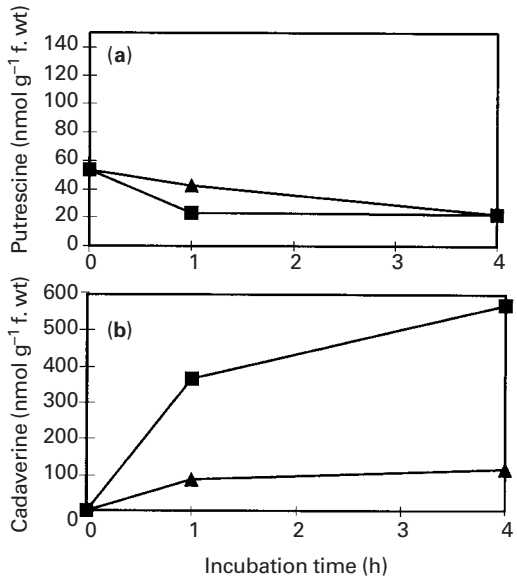

Fig. 3. Changes in content of (a) free putrescine and (b) cadaverine in 2-wk-old *Paxillus involutus* mycelia grown in liquid medium following incubation with different exogenous cadaverine concentrations (closed triangles, 0.2 mM; closed squares, 1 mM).

f. wt of spermidine respectively, data not shown), possibly because of the reduced growth rate of *Paxillus involutus* on liquid media. No spermine release was observed.

Polyamine uptake by Paxillus involutus *mycelium*

When exogenous putrescine was supplied to the liquid medium, a clear increase in the endogenous pool of putrescine (Fig. 2a) could be detected in the mycelium from the first hour of incubation. The maximum level was reached at the maximum concentration (1 mM), which displayed saturation kinetics.

Endogenous spermidine content was also affected by exogenous putrescine (Fig. 2b), since an immediate decrease occurred during the first hour, followed by a slight increase which reached its maximum after 8 h: spermine seemed not to be affected, its concentration remaining more or less unchanged throughout the incubation period (1.35 ± 0.63 nmol g^{-1} f. wt).

With regard to cadaverine uptake, a clear decrease in free putrescine (Fig. 3a) was evident from the first hour of incubation, whereas spermidine levels remained unchanged (data not shown). Free cadaverine appeared in the endogenous polyamine pool (Fig. 3b) and its level increased with time at a 1 mM concentration, whereas a stable level was reached after 1 h at a 0.2 mM concentration.

When cadaverine was supplied together with putrescine (Fig. 4), it was possible to detect an analogous trend in the endogenous content of both: from the first hour cadaverine and putrescine levels

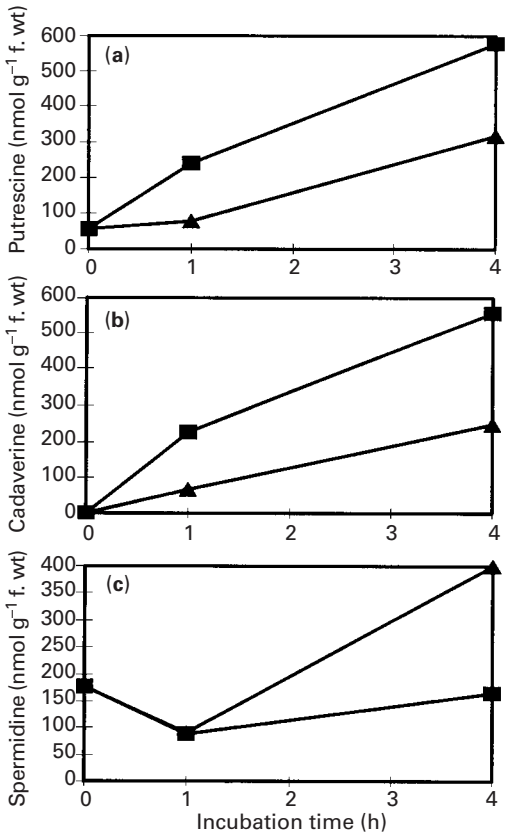

Fig. 4. Changes in content of (a) free putrescine (b) cadaverine and (c) spermidine in 2-wk-old *Paxillus involutus* mycelia grown in liquid medium following incubation with different exogenous putrescine and cadaverine concentrations (closed triangles, $0.2 + 0.2$ mM; closed squares, $1 + 1$ mM).

were practically identical and even after 4 h of incubation were still comparable. Endogenous spermidine seemed to be affected in the first hour.

Enzyme activities

Arginine and ornithine decarboxylase activities were determined in *P. involutus* mycelia, grown on solid medium, at different stages of growth (1–4 wk), both in the soluble and particulate fraction. Labelled substrate was supplied with and without the addition of 10 mM of unlabelled compound (Figs 5, 6); ODC represented the main enzyme of putrescine synthesis, while ADC activity was at least 7–8 times lower.

It should be noted that both enzymes were also present in the particulate fraction (Figs 5b, 6b), but with only 20% of the activity measured in the soluble fraction. Activity of both enzymes decreased with age. The highest ADC and ODC activities were measured in the youngest mycelium (1 wk old), whereas in the older mycelium putrescine biosynthesis decreased gradually: in the oldest mycelium (4 wk old) ADC and ODC activities were at least five times lower than in the youngest.

When exogenous unlabelled substrate was supplied together with the labelled (Fig. 5c,d), a large

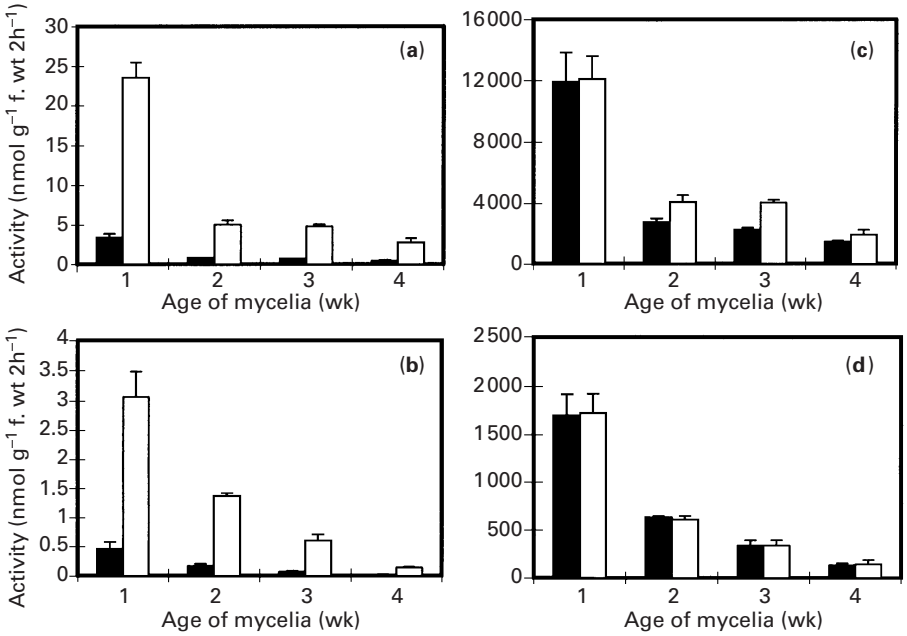

Fig. 5. ADC (closed squares) and ODC (open squares) activity in 1–4 wk-old *Paxillus involutus* mycelia grown on solid medium. Activities were determined both in soluble (a,c) and insoluble (b,d) fraction, using [^{14}C]arginine/ornithine as a tracer (a,b) and in presence of 10 mM unlabelled substrate (c,d).

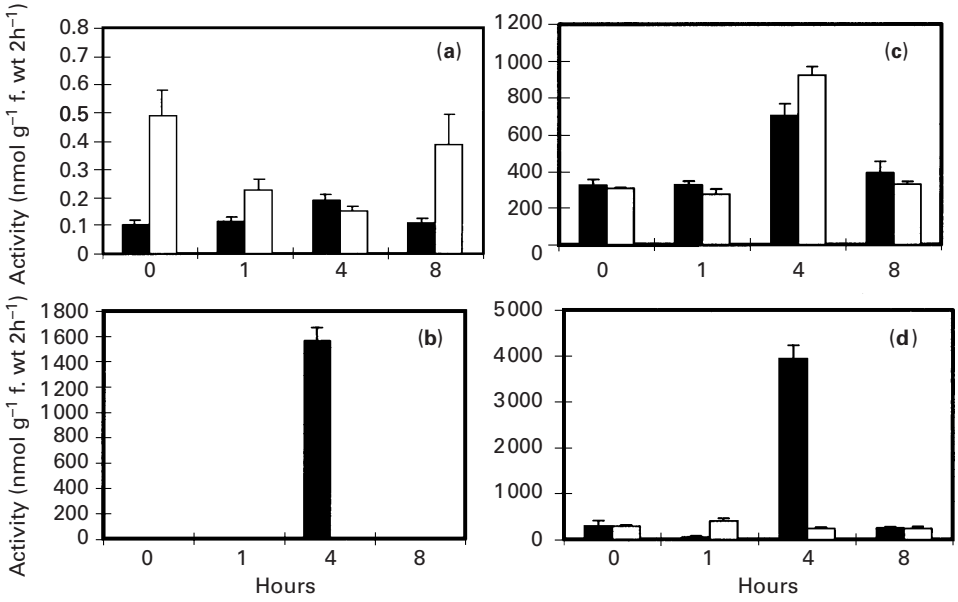

Fig. 6. ADC (closed squares) and ODC (open squares) activity in 2-wk-old *Paxillus involutus* mycelia grown on liquid medium. Activities were determined both in soluble (a,c) and insoluble (b,d) fraction, using [^{14}C]arginine/ornithine as a tracer (a,b) and in the presence of 10 mM unlabelled substrate (c,d).

increase in both ADC and ODC activities could be observed, the highest stimulation being detected in the youngest mycelium, which showed comparable levels of ADC and ODC activity. The increase in activities was *c*. 2400-fold for ADC and 500-fold for ODC in relation to the levels observed when only labelled substrate was supplied.

Saturation kinetics were reached only for soluble ADC activity at the age of 4 wk and both ADC and ODC activities were saturated in the insoluble fraction of mycelia of the same age.

In order to verify whether changes occur in putrescine biosynthetic enzymes when *P. involutus* is supplied with 1 mM putrescine for 0, 1, 4 or 8 h (Fig. 2), ADC and ODC activities were assayed in 2-wk-old mycelium grown in liquid medium. Activities were detected both in soluble and particulate fractions (Fig. 6), ODC activity once more being predominant. Activities of both soluble and insoluble ADC and ODC were 10-fold lower than those determined from mycelia grown on solid medium (Figs 5, 6). No changes could be observed in levels of

either soluble ADC or ODC in response to putrescine uptake. By contrast, a large increase in insoluble ADC activity could be observed after 4 h of incubation with 1 mM putrescine (Fig. 6b,d). Activities expressed per gram fresh weight followed the same trend as the values expressed per milligram of protein, since the ageing of mycelia did not affect protein content (data not shown).

DISCUSSION

In *Paxillus involutus*, endogenous polyamine content and the ratio of putrescine to spermidine depend on the age of the mycelium. Free and insoluble conjugated spermidine, in particular, showed contrasting patterns with respect to the age of mycelium.

Polyamine biosynthesis and release differed and were age-dependent. Young mycelium released practically no polyamines, whereas a very large release was measured from the older mycelium. It should be noted that only polyamines in free form were released, even though their conjugated forms were present at high concentrations.

This supports results observed in *Ricinus* plants (Antognoni *et al*., 1998), that conjugated polyamines are not able to cross the plasmalemma.

In particular, between weeks 3 and 4 free polyamine release was against a concentration gradient, suggesting an active release mechanism. Release of polyamines has already been demonstrated in carrot cell cultures (Pistocchi *et al*., 1987) and in the case of *Arabidopsis thaliana* protoplasts, this release occurred through potassium channels (Colombo *et al*., 1992). Furthermore, in germinating apple pollen polyamine release against concentration gradient has been observed, its ratio completely independent of endogenous content (Speranza & Calzoni, 1980).

Our results suggest different uptake transport systems for putrescine and cadaverine. No competition was observed whenever mycelia were incubated with the same concentration of the two diamines. The initial decrease in spermidine content can be explained by the activation of aminoxidases. In particular, putrescine uptake reduced ODC activity, but had almost no effect on ADC.

Yeasts, *Neurospora crassa* and *Aspergillus nidulans* (Tabor & Tabor, 1985) as well as some phytopathogenic fungi, especially rusts (Foster & Walters, 1990) do not have ADC, and synthesize putrescine by ODC only. However, other phytopathogenic fungi have active ADC (Khan & Minocha, 1989) and Zarb & Walters (1994b) gave evidences of ADC activity in *Laccaria proxima*. In *Paxillus involutus* mycelium, both ADC and ODC are present, ODC being predominant.

The presence of ADC activity was positively confirmed by arginase activity inhibition assays (data not shown). The existence of an alternative pathway for putrescine biosynthesis is in agreement with the observations of Zarb & Walters (1994a). In their work, treatment of *Paxillus involutus* mycelia with α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC, did not affect putrescine endogenous content, whereas ODC activity decreased markedly.

Activity of ADC and ODC decreases with the age of the mycelium and is related to the total putrescine content. Furthermore, in addition to cytosolic enzyme activity, activity was also found in the particulate fraction. Particulate ADC and ODC activity has been found in many higher plants; in *Paxillus involutus* both soluble and particulate ODC activity have already been found by Zarb & Walters (1994a), while in *Neurospora crassa* ODC occurs only as a cytosolic enzyme (Weiss & Davis, 1973). In this study we have demonstrated the presence of a soluble and particulate ADC activity greatly stimulated by exogenous unlabelled substrate. In the presence of 1 mM exogenous putrescine, a large increase in particulate ADC activity was observed after 4 h of incubation, suggesting different compartmentation of the enzyme and the absorbed putrescine. Although endogenous polyamine levels were high in the old mycelia, they still showed ADC and ODC activity. Active biosynthesis of polyamines which are then partly released costs energy and the benefit of this release for the fungus is not known. However, the differences between the young and the older mycelium suggest that during the ageing of the hyphae there are changes in the pattern of polyamine metabolism. If these differences also take place in a mycorrhizal stage, they could affect the physiology of young and old mycorrhizal root tips.

Mycorrhizal infection is known to decrease the rates of host root growth and cell division and ectomycorrhizal roots, by contrast with uninfected roots, have features of a slow-growing organ (Harley & Smith, 1983). The role of polyamines in mycorrhizal root growth is not known. Kytöviita & Sarjala (1997) found higher putrescine concentrations in mycorrhizal than in nonmycorrhizal Scots pine roots inoculated with *Suillus variegatus*. The role of polyamines in root growth has been studied in pear cultivars, where high putrescine concentrations were associated with lower root production (Baraldi *et al*., 1995).

El Ghachtouli *et al*. (1995) have shown that polyamines affect arbuscular mycorrhizal infection on *Pisum sativum*. They suggest that polyamines might have a role in the initial steps of the process of infection by *Glomus intraradices*. In this study, the differences in polyamine metabolism between young and old *Paxillus* mycelium might affect its different capacity to infect host roots. Polyamines are essential for microbial growth (Tabor & Tabor, 1984) and Zarb & Walters (1994a) have shown the effect of DFMO on the fungal growth of *Paxillus involutus* and *Laccaria proxima*. These considerations suggest

that polyamine release from fungal mycelium in mycorrhiza into soil might affect microbial activity in the rhizosphere.

Putrescine biosynthesis, as well as polyamine release and uptake, have been demonstrated here in *Paxillus involutus*, a common symbiont of forest trees in boreal regions. This suggests that in a symbiotic relationship the host plant could take up and translocate polyamine released by the fungus partner. However, in order to verify the release and uptake, there should be *in situ* experiments with mycorrhizal plants. If polyamines synthesized by the fungus are translocated to the host, they could constitute an additional among factors in the regulation of host growth.

REFERENCES

Abuzinadah RA, Read DJ. 1989. The role of proteins in the N nutrition of ectomycorrhizal plants. V. N transfer in birch (*Betula pendula* L.) grown in association with mycorrhizal and nonmycorrhizal fungi. *Transactions of the British Mycological Society* **91**: 473–479.

Aguilera LM, Griffiths RP, Caldwell BA. 1993. N in ectomycorrhizal mat and nonmat soils of different age Douglas-fir forests. *Soil Biology and Biochemistry* **25**: 1015–1019.

Antognoni F, Fornalè S, Grimmer C, Komor E, Bagni N. 1998. Long distance translocation of polyamines in phloem and xylem of *Ricinus communis* L. plants. *Planta* **204**: 520–527.

Antognoni F, Pistocchi R, Bagni N. 1993. Uptake competition between polyamines and analogues in carrot protoplasts. *Plant Physiology and Biochemistry* **31**: 693–698.

Bagni N. 1989. Polyamines in plant growth and development. In: Bachrach U, Heimer YM, eds. *Physiology of polyamine*, *vol. 2*. Boca Raton, FL, USA: CRC Press, 107–120.

Baraldi R, Bertazza G, Bregoli AM, Fasolo F, Rotondi A, Predieri S, Serafini-Fracassini D, Slovin JP, Cohen JD. 1995. Auxins and polyamines in relation to differential *in vitro* root induction on microcuttings of two pear cultivars. *Journal of Plant Growth Regulation* **14**: 49–59.

Caffaro S, Scaramagli S, Antognoni F, Bagni N. 1993. Polyamine content and translocation in soybean plants. *Journal of Plant Physiology* **141**: 563–568.

Chalot M, Brun A, Finlay RD, Söderström B. 1994a. Metabolism of [^{14}C]glutamate and [^{14}C]glutamine by the ectomycorrhizal fungus *Paxillus involutus*. *Microbiology* **140**: 1641–1649.

Chalot M, Brun A, Finlay RD, Söderström B. 1994b. Respiration of [^{14}C] alanine by the ectomycorrhizal fungus *Paxillus involutus*. *FEMS Microbiology Letters* **121**: 87–92.

Colombo R, Cerana R, Bagni N. 1992. Evidence for polyamine channels in protoplasts and vacuoles of *Arabidopsis thaliana* cells. *Biochemical and Biophysical Research Communication* **182**: 1187–1192.

El Ghachtouli N, Paynot M, Morandi D, Martin-Tangui J, Gianinazzi S. 1995. The effect of polyamines on endomycorrhizal infection of wild-type *Pisum sativum*, cv. Frisson (nod$^+$myc$^+$) and two mutants (nod$^-$myc$^+$ and nod$^-$myc$^-$). *Mycorrhiza* **5**: 189–192.

Finlay RD, Ek H, Odham G, Söderström B. 1988. Mycelial uptake, translocation and assimilation of N from ^{15}N-labelled ammonium by *Pinus sylvestris* plants infected with four different ectomycorrhizal fungi. *New Phytologist* **110**: 59–66.

Finlay RD, Ek H, Odham G, Söderström B. 1989. Uptake, translocation and assimilation of N from ^{15}N-labelled ammonium and nitrate sources by intact ectomycorrhizal systems of *Fagus sylvatica* infected with *Paxillus involutus*. *New Phytologist* **113**: 47–55.

Flores HE. 1991. Changes in polyamine metabolism in response to abiotic stress. In: Slocum RD, Flores HE, eds. *Biochemistry and physiology of polyamines in plants*. Boca Raton, FL, USA: CRC Press, 213–228.

Foster SA, Walters DR. 1990. The effects of polyamine biosynthesis inhibitors on mycelial growth, enzyme activity and polyamine levels in the oat-infecting fungus *Pyrenophora avenae*. *Journal of General Microbiology* **136**: 233–239.

Hadas A, Sofer M, Molina JA, Barak P, Clapp CE. 1992. Assimilation of N by soil microbial population: NH_4 versus organic N. *Soil Biology and Biochemistry* **24**: 137–143.

Harley JL, Smith SE. 1983. *Mycorrhizal symbiosis*. London, UK: Academic Press, 117–144.

Khan AJ, Minocha SC. 1989. Biosynthetic arginine decarboxylase in phytopathogenic fungi. *Life Science* **44**: 1215–1222.

Kytöviita M-M, Sarjala T. 1997. Effects of defoliation and symbiosis on polyamine levels in pine and birch. *Mycorrhiza* **7**: 107–111.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**: 265–275.

Pistocchi R, Bagni N, Creus JA. 1987. Polyamine uptake in carrot cell cultures. *Plant Physiology* **84**: 374–380.

Rabiti AL, Pistocchi R, Bagni N. 1989. Putrescine uptake and translocation in higher plants. *Physiologia Plantarum* **77**: 225–230.

Smith MA, Davies PJ. 1985. Separation and quantitation of polyamines in plant tissue by high performance liquid chromatography of their dansyl derivatives. *Plant Physiology* **78**: 89–91.

Speranza A, Calzoni GL. 1980. Compounds released from incompatible apple pollen during *in vitro* germination. *Zeitschrift für Pflanzenphysiologie* **97**: 95–102.

Tabor CW, Tabor H. 1984. Polyamines. *Annual Reviews of Biochemistry* **53**: 749–790.

Tabor CW, Tabor H. 1985. Polyamines in microorganisms. *Microbiological Reviews* **49**: 81–99.

Torrigiani P, Serafini-Fracassini D, Bagni N. 1987. Polyamine biosynthesis and effects of dicyclohexylamine during the cell cycle of *Helianthus tuberosus* tuber. *Plant Physiology* **84**: 148–152.

Weiss RL, Davis R. 1973. Intracellular localization of enzymes of arginine metabolism in *Neurospora crassa*. *The Journal of Biological Chemistry* **248**: 5403–5408.

Zarb J, Walters DR. 1994a. The effect of the polyamine biosynthesis inhibitor DFMO on the ectomycorrhizal fungus *Paxillus involutus*. *Letters in Applied Microbiology* **18**: 5–7.

Zarb J, Walters DR. 1994b. The effects of polyamine biosynthesis inhibitors on growth, enzyme activities and polyamine concentrations in the mycorrhizal fungus *Laccaria proxima*. *New Phytologist* **126**: 99–104.

Zarb J, Walters DR. 1995. Polyamine biosynthesis in the ectomycorrhizal fungus *Paxillus involutus* exposed to zinc. *Letters in Applied Microbiology* **21**: 93–95.

Zarb J, Walters DR. 1996. Polyamine biosynthesis in the ecomycorrhizal fungus *Paxillus involutus* exposed to lead. *Mycological Research* **100**: 486–488.