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SHORT NOTE

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# Effects of defoliation and symbiosis on polyamine levels in pine and birch

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**Abstract** We report the effect of ectomycorrhizal fungi (*Suillus variegatus, Paxillus involutus*) and defoliation on polyamine concentrations in pine (*Pinus silvestris*) and birch (*Betula pendula*) foliage and roots. Symbiotic root tips showed consistently higher concentrations of putrescine than non-symbiotic roots. Partial defoliation had no effect on the polyamine levels in mycorrhizal pine or birch roots. The foliage of mycorrhizal pine seedlings had lower putrescine concentrations and higher spermidine than foliage of non-mycorrhizal plants, and defoliation reversed this pattern. The response to partial defoliation differed in birch foliage: mycorrhizal status had no effect and all new growth after defoliation had higher spermidine levels than in non-defoliated birch. The potential role of polyamines in mycorrhizal symbiosis is discussed.

**Key words** Polyamines · Symbiosis · Pine · Birch · Defoliation

## Introduction

The polyamines putrescine, spermine and spermidine are found ubiquitously in living cells (Smith 1985). Their presence in fungi is also well documented, and it seems that the most abundant polyamine is spermidine (Stevens and Winther 1979), spermine being either absent or at low concentration (Davis 1996). Putrescine is a metabolic intermediate of spermidine and spermine and its pools are generally lower than those of spermidine. In pine foliage, however, putrescine seems to be the major polyamine (Sarjala and Savonen 1994; Sarjala 1996). The concentrations of spermine may be substantial in plant tissues such as pine needles (Sarjala and Savonen 1994) and roots (Sarjala 1996).

The role of polyamines is reported to include regulation of growth and development (Evans and Malmberg 1989). Polyamine biosynthesis increased during germination of fungal spores and the formation of the appressorium in the early stages of fungal infection requires polyamine biosynthesis (Reitz et al. 1995). Growth rates of several fungi have been reported to correlate positively with internal polyamine concentration (Foster and Walters 1990; Singhania et al. 1991). Ornithine decarboxylase (ODC; EC 4.1.1.17) activities are reported to correlate with the growth rate of the ectomycorrhizal fungus *Hebeloma crustuliniforme* in pure culture (Johnson and McGill 1990). Putrescine can be synthesized from ornithine by ODC and its activity correlates with polyamine biosynthesis potential (Walters 1995). In plants, the highest concentrations of polyamines are found in actively growing regions like young root tips and expanding leaves (Smith 1985).

Due to their importance in growth and developmental processes, specific inhibition of fungal polyamine biosynthesis is a novel candidate for the control of fungal pathogenic infection (Walters 1995). However, the role of polyamines in symbiotic relationships is less well known. It has been shown that externally added putrescine stimulates sugar release from the algal partner in lichen symbiosis (Legaz et al. 1985), and that in arbuscular mycorrhizae externally added polyamines increase mycorrhiza frequency (El Ghachtouli et al. 1995). In mutualistic symbioses, the plant-microbe interface is the site of exchange of elements between symbionts. In ectomycorrhizae, glucose is exported to the apoplast and subsequently imported into the fungal cell (Hampp et al. 1995), whilst amino acids (mainly glutamine) are exported from the fungal hyphae into the apoplastic space in the Hartig net (Smith and Smith 1990) and then taken up by the plant cell. As polyam-

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ines in general, and putrescine in particular, have been proposed to be involved in the maintenance of cellular homeostasis with special reference to plant mineral nutrition, we investigated whether they could be affected by the development of an ectomycorrhizal symbiosis. Firstly, we studied the effect of symbiosis on the putrescine, spermine and spermidine concentrations in pine and birch foliage and root tips by comparing mycorrhizal and non-mycorrhizal plants. Secondly, we estimated the effect of partial defoliation on polyamine levels in roots and foliage, as defoliation is likely to affect sugar transport to belowground organs and consequently affect metabolic activities at the symbiotic interface.

## Material and methods

### Plant and fungal material

Pine and birch seeds were purchased from Skog Forsk, Sävar, Sweden (*Pinus sylvestris* 184-h80, *Betula pendula* S23X8730166). The ectomycorrhizal fungi used were *Suillus variegatus* (Swartz: Fr) O. Kuntze (strain number 88.009 in the culture collection of the Department of Microbial Ecology in Lund, Sweden) and *Paxillus involutus* (Batsch) Fr. (ATCC 200175, 87.017 in the culture collection at Lund).

### Experimental methods

*Experiment 1: pine and Suillus variegatus*

Surface-sterilized pine seeds were germinated on vermiculite for 20 days. Mycorrhization with *S. variegatus* was achieved by placing the young seedlings next to an older, mycorrhizal pine in plastic 'windows' (20 cm × 20 cm × 5 mm). The plants were grown on a thin layer of peat for 10 weeks in a growth chamber with 16 h 350 μmol $m^{-2}$ $s^{-1}$/8 h light/dark period, 60/80% relative humidity and 20 °C/16 °C, respectively. At 4 months, the seedlings were mycorrhizal and were transferred into 25 × 25 × 2.5-cm observation chambers containing 200 g (fresh wt.) of a mixture of natural sand collected from a coniferous stand and commercial non-fertilized peat (1:2). The sand was microwaved twice in order to kill any natural ectomycorrhizal inoculum. The pH of the substrate at the beginning of the experiment was 3.7. No nutrients were added as *Suillus* and pine are both adapted to nutrient-poor environments. The plants were grown for a further 14 weeks in the chambers. When the plants were 6.5 months old, 40% of each needle was cut off from three mycorrhizal and three non-mycorrhizal plants. Non-defoliated plants remained as controls. Two weeks after defoliation the experiment was terminated and samples of mycorrhizal and non-mycorrhizal root tips and 5–6 top needles were frozen in liquid nitrogen and stored at −80 °C until analysed for polyamines. Because of the unequal growth rate of the symbionts in this experiment, part of the root system remained non-mycorrhizal even in the so-called mycorrhizal plants. These symbiotic and non-symbiotic roots of the same plant were harvested separately allowing comparisons within a single root system.

*Experiment 2: birch and Paxillus involutus*

Surface-sterilized birch seeds were germinated on sterile water agar for 24 days. Mycorrhization with *P. involutus* was achieved by planting the small seedlings into Petri-dishes in which the fungus was pregrown for 2 weeks in peat-vermiculite mixture with modified Melin-Norkrans (MMN) solution as nutrient source (Marx 1969). Non-mycorrhizal control plants were planted similarly without the fungal inocula. The plants were grown in a greenhouse with a 16 h/8 h light/dark period and 60/80% relative humidity. Natural light was supplemented by lamps to give 150–400 μmol $m^{-2}$ $s^{-1}$. After 72 days, 20 plants were planted in observation chambers as above. Two seedlings were placed in each chamber so that root systems grew intermingled and, in the case of the mycorrhizal plants, were interconnected by the common mycorrhizal fungus. In control treatments, a membrane barrier in the middle of the chamber prevented direct contact between plants or mycelia. At the time of planting, 20 ml of half-strength Ingestad nutrient solution (Ingestad 1979) was evenly sprayed in the chambers. The plants were grown for 46 days, during which time the mycorrhizal fungus explored most of the substrate. At this point, one of the two plants in each chamber was subjected to partial defoliation by cutting off half of each leaf with scissors. Ten ml of half-strength Ingestad nutrient solution was added to the root systems and the plants were grown for a further 19 days. The experiment was terminated by separating the two plants and collecting root and leaf material for polyamine analyses.

The root material included the apical 5–10 mm part of the youngest, actively growing roots and only young, healthy mycorrhizal root tips were collected. Extramatrical mycelium was collected where possible with forceps from the surface of the substrate before separating the plants. All plants had produced new foliage after defoliation and the youngest leaf was collected. Where this was too small to provide sufficient material for the polyamine analysis, the two youngest leaves were harvested.

### Polyamine analysis

For polyamine analyses, tissues were ground in liquid nitrogen and extracted in 5% (v/v) $HClO_4$. Soluble, free polyamines were dansylated and separated by HPLC (Merck-Hitachi) with a fluorescence spectrophotometer (Merck-Hitachi) as described by Sarjala and Kaunisto (1993).

## Results

### Polyamine concentrations in pine and *S. variegatus*

Foliage of mycorrhizal pine seedlings had lower putrescine concentrations and higher spermidine than foliage of non-mycorrhizal plants, a situation which was reversed by the defoliation treatment (Table 1). Mycorrhizal pine root tips had higher putrescine and particularly higher spermidine concentrations than the non-mycorrhizal root tips (Table 1). Defoliation did not affect putrescine concentrations in the mycorrhizal root tips, but significantly decreased those in non-mycorrhizal roots (Table 1). Root spermidine concentrations were not affected by defoliation. The root spermine concentrations were lower than those of putrescine and spermidine and probably mainly of plant origin.

In the so-called mycorrhizal plants, part of the root system remained non-mycorrhizal. The polyamine concentrations in these non-mycorrhizal root tips were similar to those in completely non-mycorrhizal plants (data not shown). This indicates that the differences between symbiotic and non-symbiotic roots was a direct consequence of the presence of fungus.

**Table 1** Mean (±SE) concentrations of polyamines (nmol/g fresh wt.) in needles and root tips of pine in symbiosis with the fungus *Suillus variegatus* and of non-mycorrhizal pine. Statistically significant differences (ANOVA followed by Tukey's test at $P<0.05$) within polyamine and sample type (root, needle) are indicated with different letters (*Def* defoliated, *Non-def* non-defoliated, *Myc* mycorrhizal, *Non-myc* non-mycorrizal)

| Sample | Putrescine | | Spermidine | | Spermine | |
|---|---|---|---|---|---|---|
| | Non-def | Def | Non-def | Def | Non-def | Def |
| Needle: Myc | 239 ±40 | 450 ±180 | 91 ±27 | 99 ±19 | 35 ±6 | 53 ±9 |
| Needle: Non-myc | 384 ±84 | 179 ± 28 | 56 ± 5 | 130 ±18 | 37 ±9 | 33 ±6 |
| Root: Myc | 370 a ±58 | 413 a ±121 | 500 a ±70 | 483 a ±67 | 20 ±5 | 24 ±6 |
| Root: Non-myc | 231 a ± 4 | 143 b ± 3 | 228 a ±56 | 199 b ±29 | 15 ±2 | 24 ±8 |

Polyamine concentrations in birch and *P. involutus*

Defoliation or the presence of mycorrhizae had no effect on the putrescine or spermine contents in birch leaves, but the spermidine contents were significantly higher in the regrowth after defoliation in both mycorrhizal and non-mycorrhizal plants (Table 2). The spermidine concentration in the non-defoliated foliage of a plant connected by mycorrhizal fungus to a defoliated plant was similar to that of non-defoliated plant without contact to a neighbour. Polyamine concentrations in mycorrhizal birch root tips were not altered by defoliation, whilst the putrescine concentrations in non-mycorrhizal root tips were reduced by defoliation (Table 2). Sufficient extramatrical mycelium was obtainable only in the defoliated material. Here, the putrescine concentrations were comparable with values measured in *P. involutus* growing in MMN media in pure culture (Table 2), and the spermidine concentrations were an order of magnitude lower in the symbiotic hyphae growing in soil.

**Table 2** Mean (±SE) concentrations of polyamines (nmol/g fresh wt.) in leaves and root tips of birch in symbiosis with the fungus *Paxillus involutus* and of non-mycorrhizal birch. Statistically significant differences (ANOVA followed by Tukey's test at $P<0.05$) within polyamine and sample type (root, leaf) are indicated with different letters (*Def* defoliated, *Non-def* non-defoliated, *Myc* mycorrhizal, *Non-myc* non-mycorrhizal, *Symbiotic mycelium* extramatrical mycelium of defoliated plants, *Non-symbiotic mycelium P. involutus* grown on MMN media in pure culture)

| Sample | Putrescine | | Spermidine | | Spermidine | |
|---|---|---|---|---|---|---|
| | Non-def | Def | Non-def | Def | Non-def | Def |
| Leaf: Myc | 24 ±11 | 28 ± 5 | 34 a ± 3 | 68 b ± 7 | 27 ±8 | 41 ±11 |
| Leaf: Non-myc | 20 ± 4 | 37 ±10 | 36 a ± 2 | 66 b ±16 | 23 ±3 | 28 ± 5 |
| Root: Myc | 58 a ± 2 | 73 a ±13 | 62 ± 2 | 67 ±28 | 2 ±1 | 3 ± 2 |
| Root: Non-myc | 39 a ±10 | 24 b ± 2 | 64 ±20 | 56 ± 7 | 8 ±3 | 7 ± 3 |
| Symbiotic mycelium | | 82 ±10 | | 53 ±13 | | 0 ± 0 |
| Non-symbiotic mycelium | | 92 ± 2 | | 767 ±48 | | 0 ± 0 |

## Discussion

The polyamine concentrations measured in the pine seedling foliage were comparable with values observed in field-grown pine trees (Sarjala and Savonen 1994) and cultivated Scots pine seedlings (Sarjala 1996). The putrescine concentrations reported here for young, expanding leaf material are low (15–50 nmol/g fresh weight) compared with those reported by Pellinen et al. (1993) for middle-aged birch leaves (100–400 nmol/g fresh wt.). As polyamine concentrations generally correlate positively with growth rate, higher values could be expected in the expanding leaves. However, the differences could be explained by different growth conditions; in particular nitrogen availability may affect the amounts of putrescine in plant tissues (Altman and Levin 1993). The relatively low amounts of putrescine in the birch foliage in our experiments are in agreement with the small amounts of nitrogen added in the nutrient solutions during the growth and experimental period.

Zarb and Walters (1994a) reported polyamine concentrations in *P. involutus* to be two orders of magnitude higher than putrescine and tenfold higher than the spermidine level reported here for the same fungus. The polyamine concentrations reported by Zarb and Walters (1994b) for *Laccaria* are also markedly higher, whilst endogenous polyamine levels in *Ophiostoma ulmi* (Biondi et al. 1993), a pathogenic fungus, were of the same order. The sensitive response of polyamines to different growing conditions and possible differences between fungal strains or species may be responsible for this variation.

Both nutrients moving from soil explored by the mycelium to the plant and the carbon allocated to the mycelium must pass through the relatively small areas of symbiotic interface in mycorrhizal roots. Consequently, mycorrhizal root tips can be expected to show higher metabolic activities than non-mycorrhizal and to involve effective buffering systems to avoid both changes in pH stat in either organism and acidification of the interface. Putrescine has been proposed to bind to excess $H^+$ (Flores et al. 1989) and the present consistently higher concentrations of putrescine in mycorrhizal root tips of both birch and pine than in non-mycorrhizal root tips are in agreement with the assumption of higher metabolic activities and buffering capacities in

mycorrhizal root tips. Externally added polyamines have been shown to increase mycorrhiza frequency between *Pisum sativum* and the endomycorrhizal fungus *Glomus intraradices* (El Ghachtouli et al. 1995). However, in contrast to our results with woody seedlings and ectomycorrhizal fungi, no difference was reported in internal concentrations of polyamines in mycorrhizal and non-mycorrhizal roots investigated in the endomycorrhizal legume (El Ghachtouli et al. 1995). The difference in polyamine levels between the symbiotic and non-symbiotic roots reported here may reflect a possible role of polyamines in the source and sink regulation and nutrient transport in mycorrhizal plants. Feray et al. (1992) have suggested that polyamines act like phytohormones and have a regulatory role in long-distance transport in plants. The symbiotic root tips did not influence the polyamine concentrations of neighbouring non-symbiotic root tips, indicating that the effect was limited to the physical presence of the fungus.

Putrescine concentrations in birch foliage were not altered by defoliation, but spermidine concentrations were significantly increased by defoliation in the newly emerged leaves in both mycorrhizal and non-mycorrhizal birch seedlings. Spermidine and spermine are generally thought to be closely associated with cell division (Flores et al. 1989), and the higher spermidine concentrations in the developing birch leaves after defoliation may be indicative of the faster growth rate of these leaves, in agreement with the general phenomenon in plants to compensate for lost photosynthetic biomass (Bloom et al. 1985). In contrast to birch leaves, the spermidine concentrations in pine needles were not affected by defoliation, and while putrescine concentrations in birch leaves showed no response to defoliation, those in pine needles seemed to increase in mycorrhizal plants and decrease in non-mycorrhizal plants after defoliation. The reponses to defoliation by conifers as slow-growing species may be expected to differ from those of fast-growing deciduous tree species.

Spermine has been shown to be involved in adventitious root growth (Jarvis et al. 1983), which is suppressed in mature mycorrhizal roots. However, the spermine concentrations reported here were low and no differences were observed between mycorrhizal and non-mycorrhizal roots.

Although further studies are necessary to establish any possible relationships between polyamine concentration and other physiological parameters in ectomycorrhizal plants, we hope that the present observations will stimulate further investigation into the role of polyamines in symbiotic systems.

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