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The influence of mycorrhizal colonization on growth in the greenhouse and on catechin, epicatechin and procyanidin in roots of *Fagus sylvatica* L.

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Abstract The influence of mycorrhizal colonization on beech (*Fagus sylvatica* L.) root tannin (procyanidin polymer) and its putative precursors catechin and epicatechin was investigated by high performance liquid chromatography. Seedlings planted in a sterile mixture of litter, compost, soil and sand were inoculated with brown beech ectomycorrhizas collected from a woodland (*Lactarius subdulcis* Bull ex Fr. × *F. sylvatica)*. The seedlings were not fertilized during the first year of growth. Nonmycorrhizal control plants showed severe nutrient-deficiency symptoms on their leaves and grew less well than mycorrhizal plants. Mycorrhizal roots contained significantly less catechin, epicatechin and procyanidin polymer than nonmycorrhizal roots. In the second year of growth, the plants were fertilized and procyanidin formation in roots was investigated. None of the fertilized plants showed mineral-deficiency symptoms. Fertilized mycorrhizal roots consistently contained significantly less catechin and epicatechin than nonmycorrhizal controls, but procyanidin polymer content varied between replicate experiments. The possible function of catechin and epicatechin in ectomycorrhizal formation is discussed.

Key words *Fagus sylvatica* · Catechin · Epicatechin · Ectomycorrhizas · Tannin accumulation

Introduction

In *Picea abies* and *Larix decidua* ectomycorrhizas, soluble phenolics such as *p*–hydroxybenzoic acid glucoside, picein, catechin and epicatechin, as well as cell-wall-bound ferulic acid, are lower than in nonmycorrhizal roots (Münzenberger et al. 1990, 1995). The lower content of cell-wall-bound phenolics possibly facilitates penetration of fungal hyphae through the middle lamella region of root tissues.

Due to their protein-complexing capacities, tannins are toxic towards bacteria and filamentous fungi and may, therefore, influence the formation of ectomycorrhizas (Scalbert 1991, 1992; Field and Lettinga 1992). Tannin deposits are frequently found in the vacuoles of outer root-cortex cells of gymnosperm ectomycorrhizas, forming the tannin layer (Duddridge and Read 1984a; Edwards and Gessner 1984). Although a tannin layer is rarely present in certain deciduous ectomycorrhizas of *Quercus* and *Fagus* (Pillukat and Agerer 1992), it is found in ectomycorrhizas of *Fagus sylvatica* from northern Germany, Eucalyptus and Hong Kong Fagaceae (Ling-Lee et al. 1977; Beyeler 1993; Tam and Griffiths 1993b).

Reports on the accumulation of tannins in the Hartig-net region of ectomycorrhizas are contradictory. In histochemical studies, tannins were found to accumulate in the root cortex of ectomycorrhizas to a greater extent than in nonmycorrhizal roots by Marks and Foster (1973) and Ling-Lee et al. (1977), whilst in later studies, no increase in tannins was detected (Piché et al. 1981; Duddridge and Read 1984a, b; Malajczuk at al. 1984).

We investigated the influence of mycorrhizal colonization on procyanidin polymer (tannin) accumulation in beech ectomycorrhizas using high performance liquid chromatography (HPLC). The soluble phenolics catechin and epicatechin, which are the putative precursors of the procyanidin polymer, were also quantified. Since formation of plant tannins is influenced by light, nutrients and tissue age (Warning et al. 1985; Scalbert and Haslam 1987), and plants deficient in nutrients accumulate more carbon-based substances like tannins, starch and lignin than supplemented plants (Bryant et al. 1987), we also investigated the influence

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of mineral fertilization on procyanidin formation in beech roots.

Materials and methods

Growth of beech seedlings

Seeds of *F. sylvatica* (Rathe GmbH, Wietze, Germany) were stratified for 5 weeks in moist and sterile sand at 4–7 °C, then germinated on vermiculite at 15–20 °C. After 4–5 weeks, groups of three seedlings with four leaves were planted in 1-l pots (18 cm high, 11 cm diameter) containing beech litter, compost, loam soil and sand (4:1:1:1, v/v/v/v) autoclaved 3 times at 24-h intervals. Inoculum consisting of excised brown beech ectomycorrhizas (*Lactarius subdulcis* × *F. sylvatica*) with a low level of white beech ectomycorrhizas [*Xerocomus chrysenteron* (Bull.) Qué l. × *F. sylvatica*], collected in natural woodland near Bremen, was put in close contact with seedling roots at planting. Mycorrhiza were determined as described by Agerer (1993). Control plants were not inoculated.

The plants were grown in a greenhouse shaded by a green weave. On sunny days, the plants received 160 mE μ^{-2} sec^{-1} and on cloudy days 62 μE m^{-2} sec^{-1} (400–700 nm). Temperature varied between 24 °C during the day and 15 °C at night, with a relative humidity of about 70%. In the first year, the plants were watered only with demineralized water.

Beech seedlings were forced to winter at the end of November by lowering the temperature in the greenhouse to 10 °C. One-year-old plants were separated in early spring by cutting the rooted soil into equal parts and replanted individually in freshly prepared sterile pot mixture (substrate and pots as before). Emergence of leaves (April) was induced by gradually raising the day temperature over 2 weeks from 10 °C to 24 °C (maximal 15 °C at night). In the second year, the plants were fertilized weekly with half-strength Knop's fertilizer supplemented with Hoagland's trace elements (100 ml per pot) (Ziegler 1991) and were grown under the same conditions as the previous year. Treatments were replicated in the first and second year and designated experiment 1 and 2, respectively. A total of 75 plants were used per treatment.

Sample collection

Samples were collected at different developmental stages of beech roots: (a) just after stratification of the seeds (radicula), (b) seedlings at the time they were planted, (c) control and inoculated plants 3 months after planting, (d) control and inoculated plants 6 months after planting, (e) 1-year-old plants 3 months after emergence of the leaves. Whole root systems of (a) and (b) were sampled. The root systems of (c), (d) and (e) were separated into two different size groups: thick roots (diameter > 0.5 mm) and fine roots (diameter ≤ 0.5 mm); only the fine roots were analysed by HPLC. The thick roots were in the secondary developmental stage whereas the fine roots were in the primary stage and, therefore, either covered with root hairs (nonmycorrhizal) or had developed mycorrhizas on their laterals (inoculated plants). For HPLC analysis, 0.6 g fresh wt. of (a) and 1.0 g fresh wt. of (b) was used whereas 0.2–0.3 g fresh wt. was used for each sample of (c), (d) and (e). The percentage of mycorrhizal root tips was determined and the mycorrhiza type checked microscopically for each sample analysed by HPLC. Root samples for HPLC analysis were weighed, frozen in liquid nitrogen and stored at –80 °C until extraction. The dry weight of the HPLC samples was determined from a dried aliquot of an homogenous root sample (60 °C, 24 h). The dry weight (DW) of the whole plant was determined to calculate the relative growth rate $R(= \ln DW_2 - \ln DW_1/t_2 - t_1)$.

Sample extraction and sample preparation for HPLC

Glassware was covered with aluminium foil to avoid photo oxidation of tannins during extraction. Roots were pulverized in liquid nitrogen, suspended in 3.5 ml of 80% acetone and homogenized for 6 min with a high-speed blender (20 000 rpm) in an ice-cooled water bath. After addition of 11.5 ml of 70% acetone, the suspensions were extracted for 1 h on a magnetic stirrer, then 2 × 30 min with 5 ml 70% acetone. The combined extracts were reduced to the aqueous phase by rotary evaporation below 40 °C, cleared by centrifugation (8000 *g*, 10 min) and prepared for HPLC analysis as follows. Silica-C18 cartridges (Sep-Pak, 500 mg, Waters-Millipore, Eschborn, Germany) were equilibrated with water. Prior to the sample application, 160 mg of an internal standard (propylgallate) was added to the cartridges. The samples were applied to the columns and washed with 2 ml water; the sample was then eluted with 2 ml of 50% acetonitrile in 0.1% *o*–phosphoric acid. Finally, the sample was analysed by HPLC (see below). Internal standards was used for quantification and to identify the signals in the chromatograms by their relative retention times.

Preparative extraction and purification of the procyanidin polymer

To identify compounds with tannin activity, acetone extracts of brown beech mycorrhizas were analysed before and after addition of gelatine. HPLC signals (detected at 280 nm) decreasing strongly after addition of gelatine are probably tannins. By means of this technique, in an earlier study we identified a major HPLC signal (Rf = 1.484, see HPLC section) whose surface decreased by 81% after the addition of gelatine (Beyeler 1993). Here, we describe the isolation and purification of a tannin corresponding to this HPLC signal. Brown beech ectomycorrhizas (*L. subdulcis* × *F. sylvatica*) were collected approximately 37 km SE of Bremen. All samples where frozen in liquid nitrogen and stored until extraction at –80 °C. Aluminium-covered laboratory goods were used to avoid photo oxidation of the tannins. A maximum amount of 4 g fresh wt. of mycorrhizas were pulverized in liquid nitrogen, suspended in 15 ml 80% acetone and homogenized for 6 min with a high-speed blender (20 000 rpm) in an ice-cooled water bath. Fifteen grams fresh wt. of the homogenates were pooled and extracted for 1 h with 300 ml and then for 2 × 30 min with 150 ml of 70% acetone in a 1-l Erlenmeyer flask. The combined extracts were reduced to the aqueous phase by rotary evaporation below 40 °C. The aqueous phase was adjusted to the initial water volume (180 ml), cleared by centrifugation (8000 *g*, 10 min) and freeze dried over night. A fluffy, light brown material was obtained, sealed under nitrogen and stored at –20 °C. For accurate results, the following purification steps had to be carried out within a few days of the extraction. Freeze-dried extract (1 g) was applied to a Sephadex LH 20 column (40 mm inner diameter, 150 mm length) (Pharmacia LKB GmbH, Freiburg i.Br., Germany) previously equilibrated with 40% acetone. The lower molecular weight components were removed by large volumes of eluent (690 ml, 40% acetone), while the tannins were adsorbed at the origin. By raising the acetone concentration (to 70% acetone) the tannins were eluted from the column as a brown-coloured band. The tannin fraction was reduced to the aqueous phase by rotary evaporation below 40 °C and freeze dried. The freeze-dried tannin fraction (150 mg) was rechromatographed with 70% acetone on a further Sephadex LH-20 column (28 mm inner diameter, 430 mm length; flow rate: 2 ml/min) previously equilibrated with 70% acetone. Fractions (13 ml) were collected, reduced to the aqueous phase by rotary evaporation below 40 °C and freeze dried. The fractions were subsequently analysed by HPLC for the presence of tannin.

Tannin characterization

An aqueous solution of purified procyanidin polymer (0.4 mg/ml) was spotted on cellulose-coated thin-layer plates and characterized by the following colour reactions: Gibb's reagent (Thompson et al. 1972), vanillin-hydrochloric acid (Swain and Hillis 1959), potassium iodate (Haslam 1982) and nitric acid (Gupta et al. 1982). In another experiment, 1 ml of an aqueous solution of purified procyanidin polymer (0.4 mg/ml) was treated 3 times with 1 ml ethyl acetate to determine if the isolated tannin is extracted by the organic solvent. The tannin concentration of the aqueous phase was determined before and after extraction by HPLC. Paper-chromatographic properties of the isolated tannin were determined as described in Thompson et al. (1972). The chromatograms were developed in freshly prepared 0.2% (w/v) $FeCl_3$ in water. Acid hydrolysis was carried out as described in Bate-Smith (1975). The hydrolysates were separated by paper chromatography to determine the anthocyanidin: 17.5 × 17.5 cm paper sheet (no. 2043 aMgl, Schleicher & Schüll, Dassel, Germany), acetic acid: conc. hydrochloric acid water (30:3:10; v/v/v) eluent (Harborne 1967). Cyanidinchloride was used as reference.

HPLC

HPLC columns from Macherey and Nagel (Düren, Germany) Nucleosil C-18, 5 μm (4.0 mm inner diameter, 250 mm length) were used with a UV detector at wavelength 280 nm. Eluents were (A) water: acetonitrile (95:5; v/v) in 0.1% *o*–phosphoric acid (w/v) and (B) water: acetonitrile (5:95) in 0.1% *o*-phosphoric acid, using gradients of 0% to 20% B over 35 min (linear gradient), 20% B for 10 min (isocratic), 20% B to 40% B (step gradient), followed by 40% B for 10 min (isocratic). Flow rate was 1.5 ml/min and the injection volume 20 μl. The HPLC signals were identified by the relative retention time (Rf) compared to the internal standard (propylgallate, retention time = 33.5 min): (+)–catechin Rf = 0.524, (–)–epicatechin Rf = 0.676, procyanidin polymer Rf = 1.484. Furthermore, the wavelength area ratios (300 nm: 280 nm) of standards were determined and used for identification: (+)–catechin was purchased from Fluka (Neu-Ulm, Germany), (–)–epicatechin from Sigma (Deisenhofen, Germany) and procyanidin polymer was isolated and purified from brown beech ectomycorrhizas (see above).

Statistical analysis

The U-test of Mann and Whitney (Sachs 1982) for the two-sided problem was used to compare the significance of differences between the medians of treatments at the level $P \leq 0.05$. To test for correlations between catechin, epicatechin or procyanidin polymer contents and the degree of mycorrhizal colonization, Spearman's rank correlation coefficient was used and tested for significant correlation at $P \leq 0.05$ (Sachs 1982). Each treatment comprised six replicate samples.

Results

Plant growth

Six months after planting, control plants developed severe symptoms of nutritional disorder, i.e. chlorotic leaves, brown spots on the leaf surface and dry, brown leaf borders, probably caused by nitrogen, potassium and magnesium deficiencies (Bergmann 1993). Mycorrhizal plants showed very few deficiency symptoms during their early growth phase when mycorrhizas became established. Fertilized plants in the second year lacked deficiency symptoms. In both fertilized and nonfertilized experiments mycorrhizal plants had higher growth rates than nonmycorrhizal plants and produced more biomass (Tables 1, 2).

Mycorrhizal formation

All nonfertilized beech seedlings inoculated with excised brown beech ectomycorrhizas (*L. subdulcis* × *F. sylvatica*) formed mycorrhizal associations. Six months after inoculation, 54–61% of total root tips were mycorrhizal (Table 2). However, the composition of mycorrhizas varied in the two experiments. Six months after inoculation, 36% brown mycorrhizas and 64% white mycorrhizas were formed on plants from experiment 1, whereas 15% white and 85% brown mycorrhizas were formed in experiment 2 (Table 2). Similarly, the mycorrhizal composition in the fertilized experiments was different in experiments 1 and 2 (Table 2). No morphological differences were observed between mycorrhizas of nonfertilized and fertilized plants. In the first year, some uninoculated plants were infected spontaneously by an unidentified ectomycorrhizal basidiomycete (hyphae bearing clamps). Six months after planting, 25% (experiment 1) and 14% (experiment 2) of the nonmycorrhizal beech plants were partially mycorrhizal (data not shown). In the second year (fertilized), 40% of all control plants had formed mycorrhizas. Only nonmycorrhizal control plants were used for growth analysis and HPLC analysis (Table 2). Roots were covered with

Table 1 Influence of mycorrhizal infection on the relative growth rate of beech grown in a sterile substrate in the greenhouse. Relative growth rates (R) and errors are indicated ($R = \ln DW_2 - \ln DW_1/t_2 - t_1$; DW_1 and DW_2: dry weights at time t_1 and time t_2, respectively). The zero time in the first year experiments corresponds to the time of inoculation and in the second year experiments to the time of leaf emergence. Plants from experiments 1 and 2 had different mycorrhiza levels (see Table 2)

		1st Year (nonfertilized)			2nd Year (fertilized)
		0–3 months	3–6 months	0–6 months	0–3 months
Experiment 1	Nonmycorrhizal	0.108 ± 0.034	0.009 ± 0.022	0.059 ± 0.016	0.050 ± 0.021
	Mycorrhizal	0.116 ± 0.027	0.023 ± 0.007	0.070 ± 0.011	0.114 ± 0.020
Experiment 2	Nonmycorrhizal	0.111 ± 0.018	0.019 ± 0.008	0.065 ± 0.008	0.062 ± 0.019
	Mycorrhizal	0.119 ± 0.020	0.030 ± 0.021	0.074 ± 0.013	0.088 ± 0.027

Table 2 Catechin, epicatechin and procyanidin polymer contents, mycorrhizal colonization of beech fine roots and total plant dry weight of beech grown in a sterile substrate in the greenhouse. Data given are medians ± median average differences of six samples. Values in a column followed by the same letter are not significantly different in the Mann-Whitney U-test at $P \leq 0.05$. The data from the first year (small letters) and the second year (capital letters) were analysed separately. In the first and second year, the treatments were replicated and designated as experiment 1 and experiment 2, respectively. The *radiculae* of experiment 2 were not analysed

Experiment 1							
Treatment	Catechin	Epicatechin	Procyanidin polymer	Plant dry wt.	Mycorrhizal colonization		
	mg/g dry wt.	mg/g dry wt.	mg/g dry wt.	g	Mycorrhizal Root tips (%)	Mycorrhiza type Brown	White
1st Year (nonfertilized)							
Radicula	22.27 ± 1.03^{a}	$1.67 \pm 0.44^{a,b}$	2.47 ± 1.14^{a}	–	0^{a}		
Seedlings	5.87 ± 0.96^{b}	1.73 ± 0.37^{b}	6.32 ± 1.55^{b}	0.247 ± 0.063^{a}	0^{a}		
3-Month nonmycorrhizal	3.33 ± 0.22^{c}	1.61 ± 0.19^{b}	$19.98 \pm 1.22^{c,d}$	0.902 ± 0.130^{b}	0^{a}		
3-Month mycorrhizal	$2.48 \pm 0.43^{c,d}$	0.75 ± 0.23^{c}	$18.57 \pm 1.80^{d,e}$	0.999 ± 0.069^{b}	34 ± 7^{b}	100%	0%
6-Month nonmycorrhizal	3.48 ± 0.24^{c}	0.95 ± 0.04^{c}	22.46 ± 3.64^{c}	$1.009 \pm 0.116^{b,c}$	0^{a}		
6-Month mycorrhizal	1.44 ± 0.08^{e}	0.73 ± 0.05^{c}	11.55 ± 1.94^{e}	1.324 ± 0.015^{c}	61 ± 7^{c}	36%	64%
2nd Year (fertilized)							
3-Month nonmycorrhizal	3.51 ± 0.17^{A}	0.71 ± 0.17^{A}	14.77 ± 0.85^{A}	1.829 ± 0.253^{A}	0^{A}		
3-Month mycorrhizal	1.77 ± 0.76^{B}	0.40 ± 0.17^{A}	14.29 ± 1.77^{A}	5.195 ± 1.180^{B}	79 ± 6^{B}	36%	64%

Experiment 2							
Treatment	Catechin	Epicatechin	Procyanidin polymer	Plant dry wt.	Mycorrhizal colonization		
	mg/g dry wt.	mg/g dry wt.	mg/g dry wt.	g	Mycorrhizal Root tips (%)	Mycorrhiza type Brown	White
1st Year (not fertilized)							
Seedlings	6.06 ± 0.46^{a}	$1.39 \pm 0.56^{a,b}$	7.51 ± 1.90^{a}	0.254 ± 0.040^{a}	0^{a}		
3-Month nonmycorrhizal	3.91 ± 0.63^{b}	1.55 ± 0.17^{a}	$27.58 \pm 2.64^{b,c}$	0.958 ± 0.051^{b}	0^{a}		
3-Month mycorrhizal	$4.71 \pm 0.27^{a,b}$	$0.63 \pm 0.17^{c,d}$	24.21 ± 3.69^{b}	$1.053 \pm 0.088^{b,c}$	24 ± 4^{b}	100%	0%
6-Month nonmycorrhizal	3.70 ± 0.41^{b}	1.65 ± 0.16^{a}	33.44 ± 2.40^{c}	$1.198 \pm 0.052^{b,c}$	0^{a}		
6-Month mycorrhizal	2.20 ± 0.28^{c}	$0.81 \pm 0.19^{b,d}$	24.34 ± 2.00^{b}	1.504 ± 0.244^{c}	54 ± 1^{c}	85%	15%
2nd Year (fertilized)							
3-Month nonmycorrhizal	3.52 ± 0.20^{A}	1.52 ± 0.38^{A}	15.07 ± 2.11^{A}	2.525 ± 0.457^{A}	0^{A}		
3-Month mycorrhizal	1.74 ± 0.14^{B}	0.25 ± 0.10^{B}	20.45 ± 0.57^{B}	4.334 ± 0.710^{B}	100 ± 0^{B}	100%	0%

root hairs and tissues of 3-month-old and 6-month-old nonfertilized control roots were irregularly stained dark, whereas the root tissue of fertilized nonmycorrhizal roots was white to yellow (not shown).

Catechin content of beech fine roots

The radicula of the beech seeds contained four times more catechin than 1-month-old beech seedling roots (Table 2). Six months after inoculation, the catechin concentration of mycorrhizal roots in both experiments was significantly lower than the catechin concentration of nonmycorrhizal roots (Table 2). The catechin content and the degree of mycorrhizal colonization of 3- and 6-month-old nonfertilized mycorrhizal beech roots were negatively correlated, i.e. catechin content decreased with increasing mycorrhizal infection (experiment 1: $n = 12$, $r_s = -0.839$, significant at $P \leq 0.01$; experiment 2: $n = 12$, $r_s = -0.671$, significant at $P \leq 0.05$). In the second year (fertilized), the catechin content of mycorrhizal roots in both experiments was half that of the nonmycorrhizal roots (Table 2).

Epicatechin content of beech fine roots

Beech roots contained between two- and sixfold less epicatechin than catechin, independent of their mycorrhizal status. Radiculae contained 13-fold times less epicatechin than catechin (Table 2). Mycorrhizal roots of both nonfertilized and fertilized plants contained half of the epicatechin content of the nonmycorrhizal roots (Table 2). The correlation coefficient for epicatechin content and degree of mycorrhizal infection of 3- and 6-month-old nonfertilized mycorrhizal roots was only significant for experiment 2 (experiment 1: $n = 12$, $r_s = -0.021$; experiment 2: $n = 12$, $r_s = -0.643$, significant at $P \leq 0.05$).

Isolation of a procyanidin polymer from beech ectomycorrhizas

A 130-g fresh wt. sample of brown beech ectomycorrhiza gave 6.6 g of freeze-dried extract, of which 6 g were used for procyanidin purification. A tannin fraction was obtained (1.06 g) from a first Sephadex LH-20 chromatography. After rechromatography on a further Sephadex LH-20 column, the early fractions contained pure high-molecular-weight tannin (51 mg; purity >95% determined by HPLC). This compound was characterized and subsequently used as an HPLC standard for procyanidin polymer quantification.

Characterization of the isolated polymer procyanindin

The UV spectrum of the isolated tannin in acetonitrile: water (1:1) showed λ_{max} at 203 and 280 nm and yielded an $E_{1\%}$ value of 136 at 280 nm. The compound gave a red coloration with vanillin-hydrochloric acid and a violet coloration with Gibb's reagent; both reactions are characteristic of proanthocyanidins. Reactions with potassium iodate and nitric acid were both negative, indicating the absence of gallotannins and ellagitannins. Paper chromatography resolved cyanidin (Rf = 0.53) from the tannin when heated with 5% hydrochloric acid, but no delphinidin could be detected. Thus the isolated tannin was a procyanidin. The isolated procyanidin could not be extracted from water by ethyl acetate, indicating that the compound was composed of at least four monomers equivalent to a M_r of > 1160 Da, as reported by Porter (1989). In two-dimensional paper chromatography, the purified procyanidin remainded at the origin. According to Haslam (1989) and Thompson et al. (1972), this chromatographic behaviour is a property of procyanidin polymers with at least 5 monomers.

Procyanidin polymer content of beech fine roots

The HPLC profiles of nonmycorrhizal and mycorrhizal beech roots were essentially identical in both the fertilized and nonfertilized experiments (data not shown). The youngest beech roots, the radiculae, contained only small quantities of procyanidin polymer (Table 2), and contents increased with plant age. Six months after inoculation, mycorrhizal roots contained significantly less procyanidin than the nonmycorrhizal roots (Table 2). The correlation coefficient for procyanidin content and degree of mycorrhizal infection of 3- and 6-month-old nonfertilized beech roots was not significant (experiment 1: $n = 12$, $r_s = -0.385$; experiment 2: $n = 12$, $r_s = -0.028$). In experiment 1, the roots of 1-year-old fertilized nonmycorrhizal and mycorrhizal plants had identical procyanidin levels. However, in experiment 2, the fertilized mycorrhizal roots contained significantly more procyanidin than fertilized control roots (Table 2).

Discussion

The mycorrhizal colonization rates obtained using excised beech mycorrhizas as inoculum are equivalent to ectomycorrhizal infection rates reported for angiosperms like oak inoculated with fungal mycelium from pure culture (for example Daughtridge et al. 1986; Tam and Griffiths 1993a). However, due to the natural origin of the inoculum used in the present study, a mixed population of brown (*L. subdulcis* × *F. sylvatica*) and white ectomycorrhizas (*X. chrysenteron* × *F. sylvatica*) was established.

Our results show that levels of the soluble phenolics catechin and epicatechin in beech roots are reduced by mycorrhizal colonization independent of the addition of fertilizer, and led us to conclude that this reduction in catechin and epicatechin is probably an important step in the process of mycorrhizal infection. Reduced catechin and epicatechin contents have also been observed following mycorrhiza establishment in *P. abies* and in *L. decidua* (Münzenberger et al. 1990, 1995).

The function of catechin and epicatechin in ectomycorrhiza is unknown. It may be that high levels of catechin and epicatechin found in nonmycorrhizal beech roots inhibit enzymes needed for ectomycorrhizal formation, and that the reduction of catechin and epicatechin levels in the root tissue leads to the activation of such enzymes. Catechin functions as an inhibitor of seed germination and plant growth, and is known to function synergistically with indole-3-acetic acid, a mechanism which is probably connected to the inhibition of indole-3-acetic acid oxydase by catechin (Buta and Lusby 1986; Feucht and Treutter 1995). Epicatechin inhibits lipoxygenase and pectate lyase, and is thought to be involved in interactions of a fungal pathogen with avocado fruits (Karni et al. 1989; Wattad et al. 1994).

The regulatory mechanisms causing the reduction in the soluble phenolics catechin and epicatechin in ectomycorrhiza are unknown, but could be based on various processes. Firstly, the polymerization rate of catechin and epicatechin may be enhanced, thus reducing the levels of corresponding monomers and enhancing procyanidin polymer content of the roots. However, the results of the nonfertilized experiments where procyanidin contents remain constant do not support such a mechanism. Secondly, catechin and epicatechin may be degraded during mycorrhiza formation. This could be caused by plant catabolism or by the action of the ectomycorrhizal fungi. Little is known about the degradation of catechin and epicatechin by plant enzymes. However, the degradation of catechin by various fungi is well documented (Galiotou-Panayotou et al. 1988; Vasudevan and Mahadevan 1990). In the case of pathogenic plant–fungal interaction, the fungus *Heterobasidion annosum* catabolizes catechin and causes a reduction in this compound in infected *Pinus* roots (Bonello et al. 1993).

The influence of the mycorrhizal fungus on the tannin content of beech roots is unclear. The experiments with nonfertilized plants indicate that the high tannin content of nonmycorrhizal beech roots is induced by nutrient deficiency of the plant tissue. Mycorrhizal infection of nonfertilized plants relieved nutrient deficiency and enhanced the growth of the plants, and thus possibly reduced the tannin content of the roots. It has been shown that *Salix aquatica* plants or leaves of tropical shrubs contain higher amounts of tannin when grown in nutrient-deficient conditions than in nutrient-rich substrates (Warning et al. 1985; Nichols-Orians 1991).

Procyanidin polymer has been detected histochemically by vanillin hydrochloric acid staining in the outer root cortex cells of brown beech ectomycorrhiza (Beyeler 1993). Since tannins accumulate in the vacuoles of plant cells (Zobel 1986; Rao 1988), ectomycorrhizal fungi probably do not come into contact with the fungi-toxic tannins present in the vacuoles of the tannin layer. Consequently, tannins may not influence mycorrhizal fungi unless cellular structures of the root tissue are disrupted. During normal development of ectomycorrhiza, root tissue is only disrupted in the senescent stage of the root–fungus interaction. In conclusion, the soluble phenolics catechin and epicatechin may have a specific role to play in the process of ectomycorrhiza formation on tree roots, whereas plant tannins may have a plant-protective role linked to their toxic effects on microorganisms.

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