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Effect of season and soil treatments on carbohydrate concentrations in Norway spruce (*Picea abies*) mycorrhizae

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Summary We studied effects of season and soil treatments (watering, acidification, liming and combinations of these treatments) on soluble carbohydrates of mycorrhizal roots of Norway spruce (*Picea abies* (L.) Karst.). Arabinose, arabitol, fructose, glucose, inositol, lactose, mannitol, pinite, quinate, raffinose, shikimate, stachyose and trehalose were identified by HPLC. Concentrations of inositol, lactose and pinite were constant throughout the year, whereas concentrations of raffinose, stachyose and trehalose were higher in winter than in summer, and concentrations of glucose, fructose and mannitol increased from February to September.

Soil acidification and liming had no effect on the annual mean concentrations of fructose, glucose, lactose, pinite, raffinose and stachyose. Liming increased quinate concentrations and decreased arabitol concentrations. Annual mean concentrations of arabinose and mannitol decreased in response to soil acidification. Annual mean concentrations of inositol increased in response to irrigation. None of the soil treatments affected the mean annual concentration of trehalose.

Keywords: carbohydrates, liming, mycorrhiza, roots, seasonal variation, soil acidification.

Introduction

Carbohydrate metabolism in mycorrhizas provides energy, reducing power and the carbon backbone for the synthesis of numerous compounds (Harley and Smith 1983). Much research on carbohydrate metabolism of mycorrhizas has focused on comparisons of free-living mycorrhizal fungi and non-mycorrhizal roots (Martin et al. 1987); however, little is known of the major pathways in the symbiotic state (Niederer et al. 1992, Rieger et al. 1992, Rothe 1993).

The carbohydrate source of mycorrhizal roots of conifers is sucrose, which is transported in the phloem to the root cells (Salzer and Hager 1993). Sucrose may be translocated from the phloem to the root cells either through a symplastic or an apoplastic pathway (Lewis and Harley 1965*a*, 1965*b*). In root cells of Norway spruce, acid invertase (EC 3.2.1.26) hydrolyzes sucrose to glucose and fructose (Salzer and Hager 1993). Ectomycorrhizal fungi such as *Amanita muscaria* (L. ex Fr.) Hooker and *Hebeloma crustuliniforme* (Bull ex St Amans) Quel, which lack invertase, depend on supplies of glucose and fructose from the host tree (Salzer and Hager 1991, 1993). Ectomycorrhizal fungi metabolize available carbohydrates into specific compounds; for example, fructose is transformed to the sugar alcohol mannitol (Söderström et al. 1988, Nowotny et al. 1993) and glucose is metabolized to the non-reducing disaccharide trehalose, the sugar alcohol arabinose and the polymeric carbohydrate glycogen (Martin et al. 1987, Niederer 1989).

Although there are more than a dozen soluble carbohydrates in ectomycorrhizas of Norway spruce only glucose, fructose (Rieger et al. 1992), sucrose (Niederer 1989, Rothe and Vogelei 1991), starch (Eichhorn 1987, Rothe and Vogelei 1991), mannitol and trehalose (Niederer et al. 1992) have been examined in detail. The pool sizes of these soluble carbohydrates depend predominantly on endogeneous cycles (Niederer 1989) and the climatic conditions of the previous year (Rothe and Vogelei 1991, Vogelei and Rothe 1991).

Because soil liming can affect the growth of Norway spruce roots and the type of fungal species infecting them (Dähne et al. 1995), we have tested the hypothesis that some soluble carbohydrate pools may also be influenced by variation in soil conditions such as water content and pH.

Materials and methods

Characterization of six experimental plots investigated at Höglwald forest

Investigations were performed on roots of 84-year-old (1991) Norway spruce (*Picea abies* (L.) Karst.) growing at Höglwald forest (48°30′ N, 11°10′ E) in the southern Bavarian forest district of Aichach, Germany, 16 km south-east of Augsburg. Six experimental plots were established in 1984 (Kreutzer and Bittersohl 1986, Kreutzer 1994). Investigations were performed exclusively on the center part of each plot, which comprised 900 m^2 and approximately 55 trees. The six plots consisted of three unlimed plots (A1, B1, C1) and three limed plots (A2, B2, C2). Liming was performed in April 1984 with finely ground Dolomite (4 Mg ha^{-1}; 881 kg Ca ha^{-1} and 519 kg Mg ha^{-1}) (Kreutzer and Bittersohl 1986). One of the unlimed plots was untreated (A1), one was irrigated with artificial acid

rain (B1; diluted H_2SO_4 equivalent to 4.1 kmol $H^+ ha^{-1}$ $year^{-1}$) and one was watered with normal rain water (C1; equivalent to 0.2 kmol H^+ ha^{-1} $year^{-1}$). Irrigation treatments began in May 1986 and continued until October 1990. The limed plots were treated in parallel: Plot A2 was limed without further treatment, Plot B2 was limed and irrigated with acid rain and Plot C2 was limed and watered with normal rain water. Irrigation was terminated in autumn 1990. The applied lime had been completely solubilized by 1988 (Plot B2) and 1990 (Plot A2), respectively (Rothe 1994).

Preparation of roots

Roots were sampled in 1991 (July 22) and 1992 (February 10, April 6, June 1, July 27 and September 21). On each of the six plots, six randomly distributed sites situated within groups of trees were selected. The sites were located 1.5 to 2 m from the base of a tree, and approximately 10 cm from a main root. Two closely adjacent soil samples were taken with a root auger (i.d. 9.1 cm) and the humus separated from the upper mineral soil (0–5 cm). On the same day, the two humus samples and the two mineral soil samples were combined to provide one sample of each. Samples were placed in plastic bags, and transported on ice to the laboratory. Roots were removed from the soil, stored on ice and fine living roots (mycorrhizal roots; diameter ≤ 1 mm) were isolated under a stereo microscope (Dähne et al. 1995). After surface drying, the mycorrhizal roots were dried at 100 °C for 48 h and weighed. Dried material (usually ≥ 100 mg) was stored in sealed plastic bags at room temperature until analyzed.

Hot water extraction of roots and dialysis of extracts

Dried fine roots were ground at room temperature with a pestle and mortar (i.d. 9 cm) and each sample was then placed in a 2-ml Eppendorf tube. Aliquots of 1.3 ml of extraction medium were added to tubes containing 75 to 100 mg of dry matter and 0.65 ml of extraction medium was added to tubes with less than 75 mg of dry matter. The exact amounts of dry matter and extraction medium were noted. The extraction medium consisted of purified distilled water to which 100 mg l^{-1} digitoxose (Sigma-Aldrich, Deisenhofen, Germany), as an internal standard, had been added. The suspension was stirred for 30 s at room temperature. The Eppendorf tubes were then incubated for 30 min at 80 °C in a shaking water bath. After 5 min of heating the vessels were briefly opened. After 15 min of heating and at the end of the extraction process, the contents of each vessel were thoroughly mixed. Samples were centrifuged at 2000 g_{av} for 20 min at 4 °C (Minifuge GL, Heraeus-Christ, Osterode, Germany). The supernatants were dialyzed with the centrifuge ultrafiltration system Centrisart® I (exclusion limit: 20 kD) (Sartorius, Göttingen, Germany) and then centrifuged at 2000 g_{av} (Minifuge GL) for 60 min at 4 °C. The volume of each ultrafiltrate was determined and stored at –20 °C for later HPLC analysis. Before HPLC analysis, the floater of each disposable ultrafiltration unit was washed three times with 2 ml of distilled water (2000 g_{av} for 15 min at 4 °C) to remove the glycerol protecting the ultrafiltration membrane.

HPLC analysis

Carbohydrate standards were obtained from commercial sources: lactose, quinate (Aldrich, Steinheim, Germany), arabitol, fructose, glucose, myo-inositol, mannitol (Merck, Darmstadt, Germany), sucrose (Boehringer Mannheim, Mannheim, Germany), shikimic acid (Roth, Karlsruhe, Germany), trehalose (Serva, Heidelberg, Germany), raffinose and stachyose (Sigma, Munich, Germany). Pinite was provided by Dr. Andreas Richter, Department of Plant Physiology, University of Vienna, Austria. Reagent-grade sulfuric acid (95–97%; 1 l = 1.84 kg) was from Merck. Purified distilled water (ROTI® Solv HPLC; Roth) was used to prepare all solutions, which were subsequently filter sterilized (Membranfilter ME 24, diameter 50 mm, pore size 0.2 µm; Schleicher and Schüll, Dassel, Germany).

Ion-exclusion chromatography was performed with a Model 6000 Lichrograph pump (Hitachi Instruments Inc., San Jose, CA), a Model 7125 sample injection valve (Rheodyne Inc., Cotati, CA), a Model T-6300 column oven (Merck), a Model 7512 differential refractometer detector (Knauer, Berlin, Germany), and a Model D-2000 Chromato-Integrator (Hitachi).

Purification of dialyzed water extracts

To avoid eventual co-elution of some carbohydrates and organic acids (cf. Togami et al. 1990), the dialyzed hot water extracts of roots were freed from organic acids on an anion exchange Ion Guard™ GA-100 (quaternized poly (styrene-divinyl-benzene)) column of 20 × 3 mm inner diameter (9 µm particle size) (Interaction Chemicals, Mountain View, CA) that was installed in the injection valve in place of the sample loop (cf. Togami et al. 1990, Schwanz and Rothe 1991). Before use, the column was equilibrated with 20 ml of 2.5 mM sulfuric acid. The dialyzed hot water extracts were then loaded and eluted in a three-step procedure. In step one, a 40 µl aliquot of dialyzed root extract containing soluble carbohydrates and organic acids was loaded into the column with a Model 705 50 µl syringe (Hamilton, Bonaduz, Switzerland). In step two, the carbohydrates were flushed from the column with HPLC-water using a Model 710 300 µl syringe (Hamilton) while the organic acids remained bound to the matrix. When the guard column was flushed six times with 50 µl of water each time the highest amounts of carbohydrates appeared in fractions 4 and 5 of the effluent. Consequently, in routine assays, the anion exchange guard column was flushed with 150 µl, then with 100 µl and the effluent saved, and finally the column was flushed with 50 µl of HPLC-water. The 100 µl fraction contained approximately 43 to 45% of the loaded amount of carbohydrates. In step three, the injection valve was turned to the injection position and organic acids were eluted from the anion-exchange guard column with 2.5 mM sulfuric acid. Carbohydrate extracts free of organic acids were stored at –20 °C and later analyzed on the same column system with 0.5 mM sulfuric acid as the eluant. The anion exchange guard column could be used for about 100 purifications.

Separation conditions for soluble carbohydrates

Carbohydrate extracts free of organic acids were chromatogra-

phed on a Polyspher® OA-KC (sulfonated poly (styrene divinylbenzene)), cation exchange column of 300 × 7.8 mm inner diameter protected by a guard column of the same material (20 × 3 mm inner diameter; Merck) (cf. Herbreteau 1992). The samples (40 µl) were loaded with a Model 705 50-µl syringe (Hamilton). The column was flushed with 0.5 mM sulfuric acid at a flow rate of 0.47 ml min^{-1}, a pressure of 5 MPa and a temperature of 75 °C.

Calibration and quantification of carbohydrates

Carbohydrates were quantified by peak heights taking 100 mg l^{-1} digitoxose as an internal standard and a calibration solution containing carbohydrate standards, including digitoxose, all at a concentration of 100 mg l^{-1}. Calibrations were performed at intervals of four to six sample separations.

Chromatograms were scanned by the Chromato-Integrator, digitized and peak heights calculated with the Chromatography Data Station Software, HPLC Manager, Version 2 (Merck –Hitachi). Carbohydrate concentrations of mycorrhizal extracts were expressed per unit mass of dry matter of fine living roots (mycorrhizal roots; mg g_{dm}^{-1}).

Quality of analyses

Separation of carbohydrates by HPLC was optimized with respect to column temperature, flow rate and molarity of the sulfuric acid eluant. Sulfuric acid at concentrations between 0 and 50 mM had no influence on the separation at 75 °C, whereas column temperature strongly influenced peak resolution. At column temperatures between 65 and 75 °C all investigated carbohydrates except sucrose separated (at a flow rate of 47 ml min^{-1}), whereas at column temperatures of 30 and 40 °C sucrose and trehalose, glucose and pinite, as well as fructose and mannitol co-eluted. Concentrations of carbohydrates were linearly correlated with refractive index in the range of 0.002 to 1.0 mg ml^{-1}; however, at concentrations below 0.008 mg ml^{-1}, arabitol and shikimic acid co-eluted. Extraction of 100 mg of dry matter with 1.3 ml of hot water yielded approximately 72% of soluble carbohydrates, a second extraction with 1 ml of hot water yielded another 21%. The extracts were not pooled because the resulting dilution gave reduced signal to noise relations. Extracting four aliquots of 100 mg of dry matter of the same sample resulted in a deviation of the mean concentration values of 2 to 6%.

Statistics

The Wilcoxon test (Weber 1986) was used to calculate significant differences between mycorrhizal roots of the humus and the upper mineral soil (0–5 cm) as well as among samples taken at different times from one of the six plots. The Mann-Whitney-U-test (Weber 1986) was used to determine pairwise significant differences between the six plots. The frequency of mycorrhizal species was determined by F. Brand and A. Taylor (Botanisches Institut, University of Munich, Munich, Germany) (cf. Dähne et al. 1995).

Results

Separation of soluble carbohydrates by ion exclusion chromatography

Under optimized conditions, soluble carbohydrates from mycorrhizal roots of Norway spruce were separated into 17 peaks by cation-exchange resin (Polyspher® OA-KC; Merck) chromatography. Of the 17 peaks, 13 were identified (Figure 1). Mycorrhizal roots also contained sucrose (elution time 13.22 min under the conditions described in Material and methods). Sucrose gave a relatively flat peak, almost half of which overlapped with the adjacent peak of glucose making its quantification uncertain. Sucrose is more completely separated from glucose at lower temperatures (65 °C), but then glucose and pinite elute too closely to be quantified. Previous investigations showed that mycorrhizal roots from Norway spruce contain an annual mean concentration of sucrose equivalent to 2.4 ± 0.25 mg g_{dm}^{-1} (Rothe and Vogelei 1991).

Estimated annual mean concentrations of some soluble carbohydrates

Among the soluble carbohydrates investigated, the highest annual mean concentrations (mg g_{dm}^{-1}) were observed for

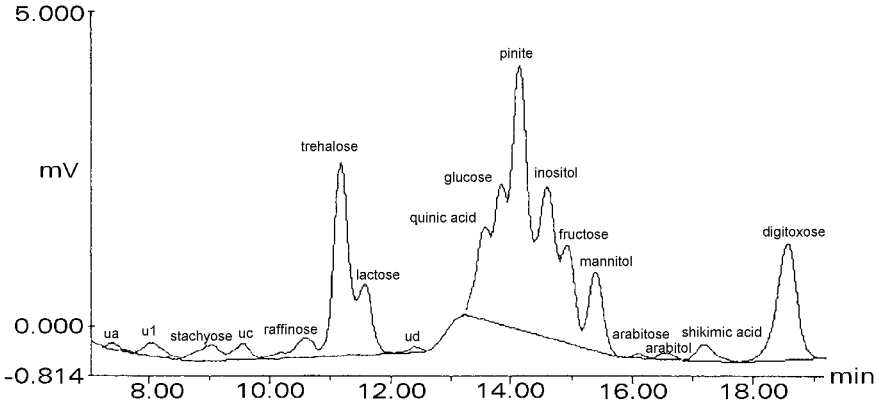

Figure 1. Separation of soluble carbohydrates from mycorrhizal roots of Norway spruce by HPLC under conditions as described in the text. Sample volume = 40 µl: sample number 4 taken on July 22, 1991 from the upper mineral soil (0–5 cm) of Plot C1 in the Höglwald experiment. Elution times (min) of separated compounds: ua (unknown a) (7.39), u1 (unknown 1) (8.10), stachyose (9.04), uc (unknown c) (9.55), raffinose (10.52), trehalose (11.15), lactose (11.58), ud (unknown d) (12.40), quinic acid (13.58), glucose (13.84), pinite (14.14), inositol (14.58), fructose (14.90), mannitol (15.39), arabinose (16.09), arabitol (16.56), shikimic acid (17.19) and digitoxose (19.15, internal standard).

Table 1. Annual mean concentrations (mg g_{dm}^{-1}) of soluble carbohydrates in mycorrhizal roots of Norway spruce from the humus and upper mineral soil (0–5 cm) from the six plots in the Höglwald.

Horizon	Plot A1	Plot B1	Plot C1	Plot A2	Plot B2	Plot C2
Arabinose[1]						
Humus (annual mean ± SD)	0.06 ± 0.03	0.03 ± 0.02	0.07 ± 0.03	0.03 ± 0.02	0.05 ± 0.03	0.04 ± 0.01
0–5 cm (annual mean ± SD)	0.08 ± 0.03	0.05 ± 0.03	0.07 ± 0.02	0.05 ± 0.03	0.06 ± 0.02	0.05 ± 0.03
Mean ± SD	0.07 ± 0.03	0.04 ± 0.02 (a1)[2]	0.07 ± 0.02 (a1)	0.04 ± 0.03	0.05 ± 0.03	0.04 ± 0.02
Arabitol						
Humus (annual mean ± SD)	0.09 ± 0.05	0.07 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02
0–5 cm (annual mean ± SD)	0.07 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
Mean ± SD	0.08 ± 0.02 (b2)	0.07 ± 0.02	0.07 ± 0.02	0.05 ± 0.01 (b2)	0.06 ± 0.01	0.06 ± 0.02
Fructose						
Humus (annual mean ± SD)	3.53 ± 1.50	4.01 ± 1.55	4.28 ± 2.12	3.54 ± 1.60	4.38 ± 1.90	4.79 ± 2.14
0–5 cm (annual mean ± SD)	3.35 ± 1.48	4.05 ± 1.77	4.38 ± 1.90	3.16 ± 1.60	4.14 ± 2.05	5.08 ± 2.25
Mean ± SD	3.44 ± 1.49	4.03 ± 1.64	4.33 ± 1.98	3.36 ± 1.59	4.26 ± 1.96	4.94 ± 2.18
Glucose						
Humus (annual mean ± SD)	2.81 ± 1.19	3.21 ± 1.26	3.55 ± 1.55	3.40 ± 1.33	3.64 ± 1.34	4.07 ± 1.75
0–5 cm (annual mean ± SD)	3.52 ± 1.56	3.84 ± 1.76	4.39 ± 1.82	3.48 ± 1.96	3.65 ± 1.62	4.37 ± 1.72
Mean ± SD	3.17 ± 1.37	3.53 ± 1.50	3.97 ± 1.66	3.44 ± 1.63	3.64 ± 1.47	4.22 ± 1.68
Inositol						
Humus (annual mean ± SD)	1.76 ± 0.26	2.11 ± 0.44	2.15 ± 0.40	1.75 ± 0.30	2.05 ± 0.53	1.93 ± 0.27
0–5 cm (annual mean ± SD)	1.59 ± 0.24 (c2)	2.41 ± 0.42	2.16 ± 0.39 (c2)	1.69 ± 0.16	1.87 ± 0.32	1.96 ± 0.32
Mean ± SD	1.67 ± 0.24 (d1)	2.26 ± 0.34	2.15 ± 0.38 (d1)	1.72 ± 0.23	1.96 ± 0.41	1.95 ± 0.26
Lactose						
Humus (annual mean ± SD)	0.66 ± 0.05	0.64 ± 0.03	0.62 ± 0.09	0.75 ± 0.11	0.68 ± 0.09	0.84 ± 0.09
0–5 cm (annual mean ± SD)	0.65 ± 0.02	0.65 ± 0.10	0.59 ± 0.06	0.71 ± 0.10	0.67 ± 0.05	0.74 ± 0.09
Mean ± SD	0.65 ± 0.03	0.64 ± 0.03	0.61 ± 0.07	0.72 ± 0.09	0.68 ± 0.07	0.78 ± 0.09
Mannitol						
Humus (annual mean ± SD)	0.68 ± 0.30	0.44 ± 0.15	0.46 ± 0.17	0.64 ± 0.26	0.48 ± 0.07	0.78 ± 0.21
0–5 cm (annual mean ± SD)	0.96 ± 0.37	0.80 ± 0.13	0.61 ± 0.18	0.69 ± 0.12	0.56 ± 0.21	1.05 ± 0.31
Mean ± SD	0.82 ± 0.32 (d1)	0.52 ± 0.15 (d1)	0.51 ± 0.19	0.72 ± 0.16	0.59 ± 0.09	0.91 ± 0.22
Pinite						
Humus (annual mean ± SD)	4.27 ± 0.46	4.45 ± 0.59	4.15 ± 0.40	4.12 ± 0.50	4.55 ± 0.97	4.29 ± 0.24
0–5 cm (annual mean ± SD)	3.90 ± 0.53	4.53 ± 0.86	4.30 ± 0.59	4.19 ± 0.53	4.37 ± 0.65	4.33 ± 0.42
Mean ± SD	4.08 ± 0.42	4.48 ± 0.59	4.22 ± 0.41	4.15 ± 0.49	4.46 ± 0.75	4.31 ± 0.27
Quinic acid						
Humus (annual mean ± SD)	1.01 ± 0.34 (e3)	1.11 ± 0.53	1.27 ± 0.41	1.80 ± 0.49 (e3)	1.61 ± 0.52	1.73 ± 0.33
0–5 cm (annual mean ± SD)	1.02 ± 0.11	0.96 ± 0.14	1.13 ± 0.35	1.19 ± 0.09	1.25 ± 0.46	1.51 ± 0.43
Mean ± SD	1.00 ± 0.23 (f2)	1.05 ± 0.34	1.20 ± 0.38	1.60 ± 0.37 (f2)	1.25 ± 0.47	1.62 ± 0.33
Raffinose						
Humus (annual mean ± SD)	0.55 ± 0.46	0.54 ± 0.55	0.64 ± 0.60	0.60 ± 0.63	0.54 ± 0.58	0.66 ± 0.63
0–5 cm (annual mean ± SD)	0.54 ± 0.48	0.51 ± 0.45	0.56 ± 0.57	0.56 ± 0.55	0.47 ± 0.47	0.46 ± 0.42
Mean ± SD	0.54 ± 0.45	0.52 ± 0.50	0.60 ± 0.59	0.58 ± 0.59	0.51 ± 0.52	0.56 ± 0.52
Shikimic acid						
Humus (annual mean ± SD)	0.38 ± 0.15	0.30 ± 0.14	0.38 ± 0.16	0.39 ± 0.14 (g2)	0.38 ± 0.14	0.52 ± 0.25
0–5 cm (annual mean ± SD)	0.31 ± 0.13	0.31 ± 0.13	0.30 ± 0.11	0.27 ± 0.08 (g2)	0.32 ± 0.12	0.46 ± 0.19
Mean ± SD	0.34 ± 0.14	0.30 ± 0.13	0.34 ± 0.14	0.33 ± 0.11	0.35 ± 0.13	0.49 ± 0.22
Stachyose						
Humus (annual mean ± SD)	0.81 ± 0.67	1.08 ± 1.33	1.12 ± 1.33	1.47 ± 1.71	1.21 ± 1.38	1.52 ± 1.75
0–5 cm (annual mean ± SD)	1.05 ± 1.30	1.06 ± 1.37	1.28 ± 1.62	1.41 ± 1.83	1.02 ± 1.19	1.00 ± 1.22
Mean ± SD	0.94 ± 1.12	1.07 ± 1.35	1.20 ± 1.48	1.43 ± 1.78	1.12 ± 1.29	1.26 ± 1.49
Trehalose						
Humus (annual mean ± SD)	0.85 ± 0.33	0.93 ± 0.28	1.22 ± 0.53	1.26 ± 0.52	0.86 ± 0.26	1.06 ± 0.32
0–5 cm (annual mean ± SD)	0.96 ± 0.37	1.05 ± 0.47	1.15 ± 0.57	1.28 ± 0.61	0.95 ± 0.27	1.34 ± 0.50
Mean ± SD	0.91 ± 0.32	0.99 ± 0.37	1.19 ± 0.48	1.26 ± 0.47	1.91 ± 0.25	1.20 ± 0.38

[1] Arabinose, lactose, quinic acid and stachyose samples were taken in July 1991 and February and September 1992; the remaining carbohydrate samples were taken in July 1991 and February, April, June, July and September 1992.

[2] The same lower case letters in brackets together with a number indicate that two groups of values differ at a significance level corresponding to the number given, (1): $\alpha = 0.05$, (2): $\alpha = 0.01$ and (3): $\alpha = 0.001$.

fructose (4.06 ± 1.81), glucose (3.66 ± 1.55) and pinite (4.28 ± 0.49). Intermediate concentrations were observed for inositol (1.95 ± 0.31), stachyose (1.42 ± 1.42), quinic acid (1.23 ± 0.35) and trehalose (1.08 ± 0.38 mg g_{dm}^{-1}). Low concentrations were observed for lactose (0.68 ± 0.063), mannitol (0.68 ± 0.19), raffinose (0.55 ± 0.53) and shikimic acid (0.36 ± 0.15 mg g_{dm}^{-1}). The lowest annual mean concentrations were estimated for arabitol and arabinose (0.05 ± 0.025 and 0.07 ± 0.0017 mg g_{dm}^{-1}) (Table 1).

Seasonal courses of the concentrations of some soluble carbohydrates

Soluble carbohydrates can be grouped into four classes based on their seasonal variation in concentration in mycorrhizal roots. In Class I, the concentrations of carbohydrates remained constant during the investigated period; e.g., inositol (1.95 ± 0.31), lactose (0.68 ± 0.06) and pinite (4.28 ± 0.49 mg g_{dm}^{-1}). In Class II, concentrations were higher in winter than in summer; e.g., raffinose (February: 1.63 ± 0.53; July: 0.18 ± 0.20), trehalose (February: 1.67 ± 0.56; July: 0.88 ± 0.33) (Figure 2) and stachyose (February: 3.17 ± 0.86; July: 0.15 ± 0.097 mg g_{dm}^{-1}). In Group III, concentrations increased from midwinter to the end of summer; e.g., fructose (February: 2.63 ± 0.74; September: 7.08 ± 1.43 mg g_{dm}^{-1}) glucose (February: 2.05 ± 0.44; September: 6.24 ± 1.45 mg g_{dm}^{-1}), and mannitol (February: 0.47 ± 0.24; September: 0.95 ± 0.49 mg g_{dm}^{-1}) (Figure 3). Between July 1988 and July 1989, sucrose concentrations increased in a similar manner to the glucose and fructose concentrations in 1992 (Rothe and Vogelei 1991). In Group IV, concentrations were highest in spring and lowest in summer; e.g., shikimic acid (April: 0.51 ± 0.18; July: 0.12 ± 0.048 mg g_{dm}^{-1}) (Figure 4).

Concentrations of soluble carbohydrates in mycorrhizas of the humus and the upper mineral soil

The concentration of fructose paralleled that of glucose irrespective of the time of investigation and whether mycorrhizal roots came from the humus or the upper mineral soil (0–5 cm) (Figure 3). Concentrations of arabinose, arabitol, inositol, lactose, mannitol, pinite, quinic acid, raffinose, shikimic acid, stachyose and trehalose in roots from the humus were similar to the corresponding concentrations in roots from the upper mineral soil (0–5 cm) (Table 1).

For the limed plot (A2), the amount of shikimic acid in mycorrhizal roots from the humus (0.39 ± 0.14 mg g_{dm}^{-1}) was significantly higher than in mycorrhizal roots from the upper mineral soil (0–5 cm) (0.27 ± 0.08 mg g_{dm}^{-1}) (Table 1). At the acid-treated plot (B1), the quantity of mannitol in mycorrhizal

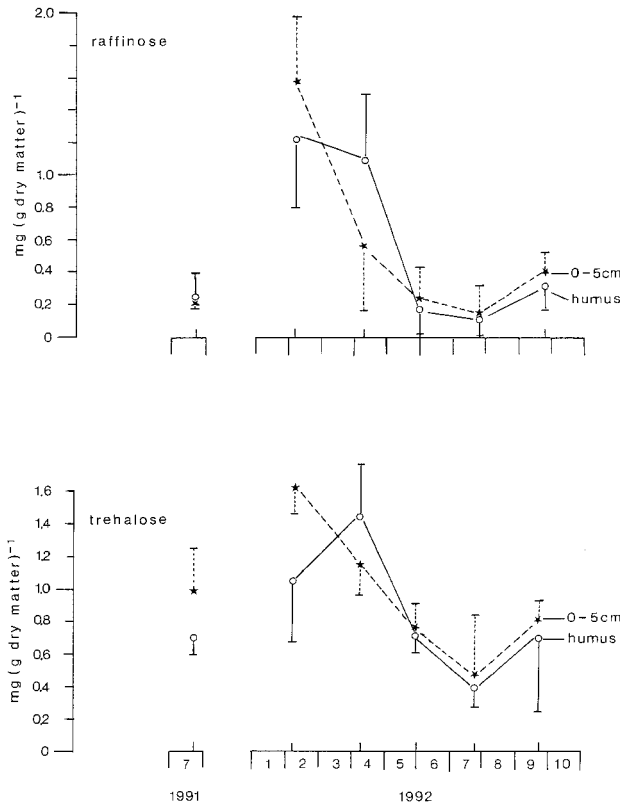

Figure 2. Seasonal concentrations (mg g_{dm}^{-1}) of raffinose and trehalose from mycorrhizal roots of the humus and the upper mineral soil (0–5 cm) of the untreated plot (A1) in the Höglwald experiment. Bars indicate standard deviations (n = 6).

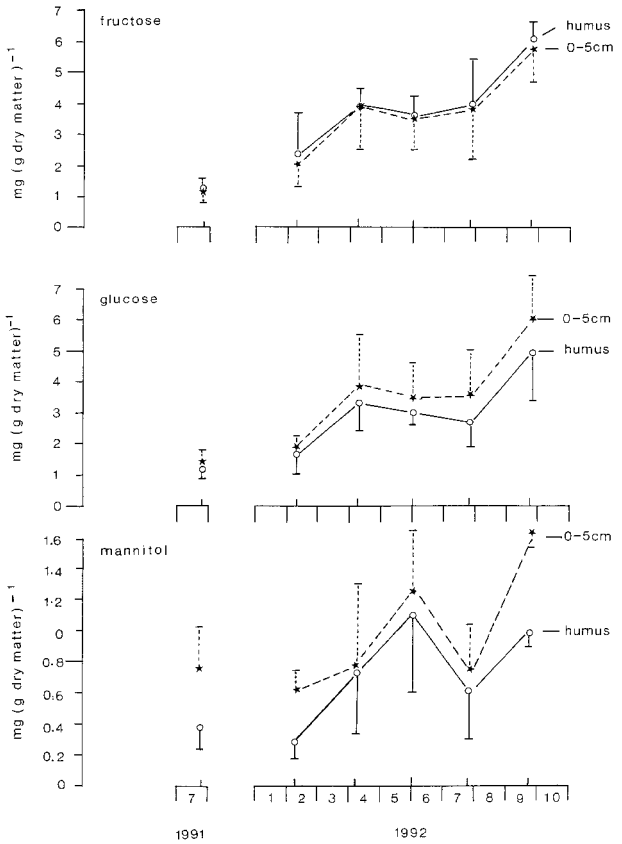

Figure 3. Seasonal concentrations (mg g_{dm}^{-1}) of fructose, glucose and mannitol in mycorrhizal roots from the humus and the upper mineral soil (0–5 cm) of the untreated plot (A1) in the Höglwald experiment. Bars indicate standard deviations (n = 6).

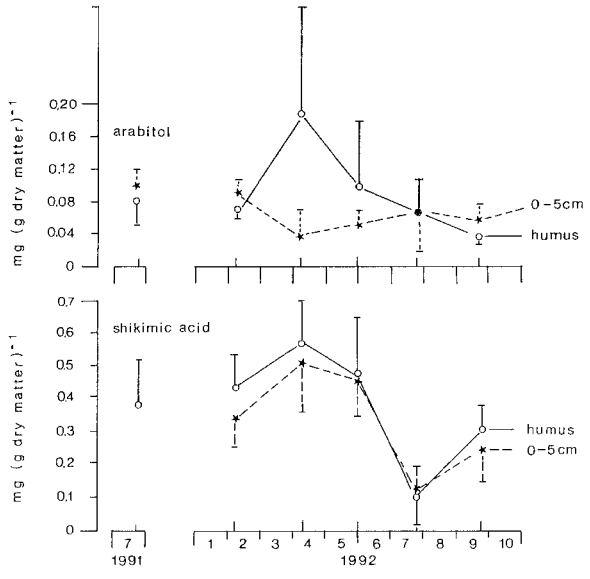

Figure 4. Seasonal concentrations (mg g_{dm}^{-1}) of arabitol and shikimic acid in mycorrhizal roots from the humus and the upper mineral soil (0–5 cm) of the untreated plot (A1) in the Höglwald experiment. Bars indicate standard deviations ($n = 6$).

roots from the humus was significantly lower (0.44 ± 0.15) than that of mycorrhizal roots from the upper mineral soil (0–5 cm) (0.80 ± 0.13 mg g_{dm}^{-1}) (Table 1).

Influence of soil acidification, liming and compensatory liming on some carbohydrate pools

Differences in chemical properties of the humus of the six experimental plots had no influence on the annual mean concentrations of fructose, glucose, lactose, pinite, raffinose and stachyose (Table 1). Liming, on the other hand, increased the concentrations of quinate in mycorrhizal roots from the humus from 1.00 ± 0.23 to 1.60 ± 0.37 mg g_{dm}^{-1}. In the upper mineral soil (0–5 cm), soil watering increased the annual mean inositol concentrations in mycorrhizal roots from 1.67 ± 0.24 to 2.15 ± 0.38 mg g_{dm}^{-1}.

Among the fungal carbohydrates, trehalose concentration was not affected by any of the soil treatments (Table 1), whereas the annual mean arabinose concentrations decreased in response to soil acidification from 0.07 ± 0.03 to 0.04 ± 0.02 mg g_{dm}^{-1}. The annual mean arabitol concentrations decreased in response to liming from 0.08 ± 0.02 to 0.05 ± 0.01 mg g_{dm}^{-1}.

Discussion

The concentrations of inositol and pinite remained constant throughout the study. Pinite is synthesized from inositol and is considered to be a carbohydrate reserve with a long half-life (Schilling et al. 1971, Diamantoglou 1974). A constant pinite concentration indicates that mycorrhizas were constantly supplied with carbohydrates. Concentrations of the carbohydrates raffinose and stachyose, which are both involved in the biochemistry of frost resistance (Hida et al. 1962, Kandler and Hopf 1980, Hinesley et al. 1992), increased markedly during the relatively mild winters of 1991 and 1992, indicating that their production was triggered not by low temperatures, but by an endogeneous cycle.

The concentrations of fructose and glucose increased steadily from midwinter to the end of summer. The molar ratio of fructose and glucose was approximately 1, indicating that both metabolites originate from the hydrolysis of sucrose. Between May 1988 and June 1989, sucrose concentrations in mycorrhizal roots increased in parallel with the concentrations of glucose and fructose in 1992 (Rothe and Vogelei 1991). Increasing growth rates of the trees during the study period (Röhle 1994) also indicate optimum carbohydrate synthesis and allocation from the crown to the stem and roots.

The carbohydrates quinic acid and shikimic acid are intermediates of the shikimate pathway. The concentration of shikimate was low when the concentration of quinate was high and *vice versa*, perhaps indicating a negative feedback mechanism. Based on the low mean concentration of shikimic acid in mycorrhizal roots compared with coniferous needles (Chung and Barnes 1977, Cranwick et al. 1987), we conclude that shikimic acid does not function as a reserve carbohydrate in Norway spruce roots.

In mycorrhizas, the soluble carbohydrates arabinose, arabitol, mannitol and trehalose are specific to ectomycorrhizal fungi (Hult and Gartenbeck 1979, Martin et al. 1987, Niederer 1989), which synthesize arabitol and trehalose from glucose and mannitol from fructose (Hult and Gartenbeck 1979). Fructose concentrations of the host and mannitol concentrations of the fungi were strongly correlated over the investigated period, confirming that mannitol is synthesized from fructose. Although plant glucose is considered a precursor of fungal arabitol and trehalose, plant glucose concentrations were not correlated with fungal arabitol and trehalose concentrations.

Neither soil differences between the humus and the upper mineral soil, nor differences among the six experimental plots (cf. Rothe 1994) influenced the pool sizes of the host-specific carbohydrates fructose, glucose, lactose, pinite, raffinose and stachyose. However, these findings do not preclude the possibility that soil chemistry influences the metabolism of glucose in mycorrhizas. Root growth, which involves the glucose-consuming synthesis of cell wall material, was strongly influenced by soil pH. At pH values of ≥ 4.5, mycorrhizal root growth increased considerably, whereas it decreased at pH values ≤ 4.5 (Nowotny et al. 1998). We hypothesize that the glucose pool of the root cells is fed by way of the symplast, whereas the glucose needed for the synthesis of cell wall material comes from the apoplast and is, therefore, influenced by a change in apoplastic pH.

Inositol is presumed to originate from root cells of mycorrhizas, and is probably a precursor of pinite and glucuronate. Its concentration increased in mycorrhizal roots from the humus and the upper mineral soil (0–5 cm) in response to soil watering, whereas the pinite concentration remained unaltered, indicating that the inositol pool does not influence the pinite pool. It is not known whether the inositol pool influences the glucuronate pool.

Quinate, which is a metabolite of the shikimate pathway, increased in response to liming in mycorrhizas from the hu-

mus. Because the shikimate pathway synthesizes aromatic amino acids, increased quinate concentrations may indicate an increased synthesis of aromatic amino acids. This concept is in line with the observation of an increased synthesis of soluble proteins in Norway spruce mycorrhizas after liming of the Höglwald soil (Dähne et al. 1995).

Trehalose concentrations were not affected by the soil treatments, but arabinose and mannitol concentrations decreased significantly in response to soil acidification (B1:C1). This response can be explained by an increased infection of root tips by the fungi *Elaphomyces* sp. (B1:C1 = 13:8), *Hygrophorus* sp. (B1:C1 = 5:0), *Russula ochroleuca* (Pers.) Fr. (B1:C1 = 60:33) and *Xerocomus badidus* (Fr.) Kühn. Gilbert (B1:C1 = 7:5) (F. Brand and A. Taylor, personal communication) at low pH. Arabitol concentrations, on the other hand, decreased following liming (A2:A1) as a result of increased pH leading to increased infection of root tips with mycorrhizal fungi such as *Amphinema byssoides* (Pers.) J. Erikss. (A2:A1 = 10:0), *Tuber puberulum* Berk. & Br. (A2:A1 = 12:0) and different mycorrhizas such as *Piceirhiza nigra* (A2:A1 = 35:0) and *Piceirhiza oleiferans* Agerer 1987–1997 (A2:A1 = 3:0) (F. Brand and A. Taylor, personal communication).

On all plots, mannitol concentrations were approximately 35% lower in mycorrhizal roots from the humus compared with mycorrhizal roots from the upper mineral soil (0–5 cm). This difference was probably caused by the different preferences of mycorrhizal fungi (e.g., *Amphinema byssoides* and *Tylospora* sp.) and mycorrhizas (e.g., *Piceirhiza nigra*) for humus and upper mineral soil (e.g., *Elaphomyces* sp. and *Russula ochroleuca*), respectively (F. Brand and A. Taylor, personal communication).

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