VARIATION OF CARBOHYDRATES AND LIGNIN IN HYBRID ASPEN (*Populus tremula* × *P. tremuloides*) ON ALKALINE SOIL

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Received January 10, 2011

Hybrid aspen plantations established on soils with different pH and chemical composition were studied. At higher pH and Ca, K, Mg and P in the soil of a former arable land, influenced by alkaline dust pollution long before plantation establishment, an inhibition of growth, diameter at breast height, as well as of the annual growth was observed, compared to unpolluted plantations. The differences in hybrid aspen leaves, branches and stems from trees growing under optimal growth conditions and those from polluted areas consisted in higher sucrose, starch, glucose, fructose and hemicelluloses contents, and lower lignin and cellulose contents in the trees from polluted plantations. The relatively high C/N ratio was associated with the lower N concentration and height growth of the trees growing in the polluted area. This finding suggests that, although no differences occurred in the total C concentration among trees from different plantations, an altered partitioning was established between the compartments of trees, as to starch and total soluble carbohydrates.

Keywords: hybrid aspen, cellulose, lignin, hemicellulose, non-structural carbohydrates, carbon, nitrogen, growth, soil

INTRODUCTION

Aggressive afforestation, including the establishment of short-rotation forest plantations with highly productive species, such as those of the *Populus* genus, is considered an option for increasing the forest cover.[1] Although it is not one of the most commercially valued trees, Eurasian aspen (*Populus tremula*) and its North-American counterpart *P. tremuloides* and their hybrids (*Populus tremula* × *P. tremuloides*) have very modest requirements for climatic conditions, growing well under relatively extreme growth conditions, such as reclaimed surface mines,[2,3] reclaimed opencast oil shale mines,[4] as well as under the impact of elevated O_3, SO_2 and industrial fly ash.[5,6] As aspens are relatively poor in lignin and rich in carbohydrates and have fibres narrow in diameter and with thin walls, their wood is amenable to many kinds of chemical or mechanical pulping.[7,8]

Recently, the forest industry has shown renewed interest in utilising aspen not only for pulp production, but also for bioenergy production, so that a significant increase in the use of aspen can be expected.[9-11]

The interest in plantations has been increasing and the establishment of short-rotation forest plantations is widely recommended at present.[12] Although aspens are a widely distributed species in different climatic conditions, they need relatively fertile soils for fast growth. The mineral composition of the soils and the conditions for mineral nutrition are essential for many aspects of hybrid aspen physiology, being considered an important factor for the growth of trees and for their wood quality.[13-16]

As tree biomass and wood formation represent an ecologically essential carbon sink, carbon cycling and carbohydrate metabolism are important in these

processes.[17-19] Plant non-structural carbohydrates, including starch and hexoses, are primary intermediate products of carbon assimilation, which can be stored and used to meet future growth and metabolism demands.[20,21] Sucrose is a primary translocatable carbohydrate from the source tissues to non-photosynthetic tissues. The carbon delivered by sucrose transport is then utilised as an energy source and as a skeleton for building new molecules during growth and respiration.[22] A major proportion of carbon is used for cambial growth and differentiation of secondary xylem. Once carbon is allocated to secondary wall cellulose, hemicellulose or lignin, it is permanently immobilised for the lifetime of the tree.[21,23]

The ratio of cellulose, hemicellulose and lignin in a biomass feedstock is a very important criterion in determining its suitability as an economically viable feedstock, and in deciding on the optimum pathway for its conversion. Poplar species and hybrids have[24] contents of cellulose ranging from 42 to 49%, of hemicellulose – from 16 to 23%, and of total lignin – from 21 to 29%. For hybrid aspen stemwood, much lower lignin (10.5-11.7%) and higher cellulose contents (57-60.2%) were established.[15] However, any factor affecting the physiology and biochemistry of a tree can change the ratios of cellulose, hemicellulose and lignin and, generally, wood quality.[25-27] Numerous studies have reported relationships between lignification and soil chemical composition.[19,28,29] Both deficiency and abnormally high concentrations of nutrients in the soil can cause abnormalities in wood formation.[30,31] Maintaining nutrition to achieve the highest possible growth rates is more critically important for hybrids grown under intensive management regimes, mainly to produce pulp.

Research on different aspects of wood formation has been, so far, predominantly focused on the influence of N[29,32] and K.[33] When poplar trees were grown under low K^+ and Ca^{++} regimes, the cambial activity, as well as the seasonal rate of wood increment and the vessel size, were significantly reduced.[34] In hybrid aspen, Ca deficiency is also responsible for wood deformation, which includes a smaller diameter of the xylem vessel, shorter fibre length and reduced levels of S units in lignin.[35] The investigations on the effects of mineral nutrition on wood formation should be continued, because the mechanisms are not sufficiently clear.

In recent years, the reduction in lignin contents and the increase in cellulose content in aspen have aroused special interest. The modification of the genes encoding enzymes along the lignin biosynthetic pathway[36] or hybridisation may give trees with low lignin and increased cellulose contents.[37]

Since 1999, about 700 ha of industrial hybrid aspen plantations have been established on abandoned agricultural lands in different regions of Estonia.[38] Growth dynamics in Estonian plantations has followed a similar trend as that of Scandinavian hybrid aspen plantations,[14] where growth data on the whole rotation period are available, and the average annual volume increment has reached values as high as 12-25 m^3 ha^{-1} $year^{-1}$ during the 25-year rotation.[9,11]

The aim of this study was to analyse the effects of different soil conditions on the partitioning and concentrations of soluble and structural carbohydrates (cellulose, hemicelluloses) and lignin, in different compartments of hybrid aspen planted on abandoned agricultural lands, in an unpolluted area and in an area influenced by cement dust pollution in North Estonia. Our primary objective was to assess the dependence of structural carbohydrates, cellulose, hemicelluloses and lignin in hybrid aspen on the quality of soils and on the growth rate of trees.

MATERIALS AND METHOD

Study area

The study was conducted with two hybrid aspen (*Populus tremula* × *P. tremuloides* Michx.) plantations established on former agricultural lands in North Estonia. The plantations were established with 1 year-old micropropagated plants belonging to clones C05-99-10 and C05-99-34, with an average rate of 1300 trees ha^{-1} (clone identification numbers according to the Finnish plant Production Inspection Centre).

Climatically, the study area belongs to the Atlantic continental region, where the influence of the Baltic Sea is felt. The mean annual temperature is of 4.9 ºC, annual amount of precipitation – 550 mm, dominating winds blowing from South and South-West at a mean velocity of 5.2 m s^{-1} (data from the Estonian Meteorological and Hydrological Institute). The soils in the plantations are well-drained gravelly

mineral soils developed on stony calcareous till on Ordovician limestone.

Plantation No. 1 (Kunda, 59º29' N, 26º34' E) was established (1999) on a territory influenced over 40 years by a cement plant in the town of Kunda, North Estonia. The plantation is located at a distance of 3 km E from the emission centre. The emission from the cement plant contained 87-91% of technological dust and 9-13% of gaseous pollutants (SO_2, NO_x, CO etc.).[39] Dust pollution emitted for a long time from the cement production contained many components, among which the following are predominant: 40-50% CaO; 12-17% SiO_2; 6-9% K_2O; 4-8% SO_3; 3-5% Al_2O_3; 2-4% MgO, etc. The water solution of dust from the electric filters had[39] pH values from 12.3 to 12.7. Dust emission from the cement plant was extremely high between 1990-1992, of 80-100 Mt per year.[40,41]

In 1996, the emission of cement dust from the plant decreased notably, thanks to the installation of efficient filters, being now lower than the permitted quantity (421 t $year^{-1}$).[42] When the plantation was established (1999), the dust emission from the cement plant had practically stopped.

In the region of the plantation, on a former arable land, the typical soils are Calcaric Cambisols – according to the World reference base for soil resources.

Plantation No. 2 (Rapla, 58º53' N, 24º41' E) was established (2000) under similar climatic and edaphic conditions, on an unpolluted area, at a distance of about 120 km W from the first plantation, on former arable land, where the soils were Chromic Cambisols – according to the World reference base for soil resources.

Plant material analyses

The investigations were carried out in August 2010, when shoot growth ceased and the leaves were fully expanded. From both plantations, 6 visually average in height model trees were selected for morphological measurements, as well as for collecting samples for chemical analyses. The average stem diameter at breast height (DBH, cm), height (H, m) and current annual height increment (HI, m) of trees within the plantation were measured. The initial height of hybrid aspens at planting was of 45 cm.[54]

The leaves and branches, collected evenly through the crown and stem wood disks (10 cm in length), were taken from 1.3 m height. The samples were dried at +70 ºC and ground in a laboratory mill (Tecator Cyclotec) with a screen that yielded particle size <0.5 mm. After grinding, the dried plant material, the wood components, the carbohydrates and N and C were determined.

The concentration of total nitrogen (%) in the plant samples was determined by the standard Kjeldahl procedure. Carbon (%) was measured by the dry combustion method, on a Vario MAX CNS elemental analyzer (Elementar, Germany).

The leaves were fixed in 96% boiling ethanol for 4 min. Total soluble carbohydrates (TSC, %) were determined with the methods recommended by Hansen and Møller[43] and Häikiö *et al.*[44] The dried plant material was ground to powder in a ball mill (MM 2000; Retsch, Vienna, Austria). The powdered plant material (40 mg) was extracted with 3 mL of 80% ethanol. The samples were incubated at 60 ºC for 5 min and centrifuged (2000 *g*, 4 min). The supernatant was collected and extraction was repeated 4 times. An aliquot (400 µL) of combined supernatants was added to 2 mL of anthrone reagent (2 mg anthrone in 1 mL of 72% sulphuric acid). Each sample was boiled for 11 min and cooled, and absorbance was determined at 630 nm.[43] The combined residues were saved for starch (ST) determination. The starch (ST) residue was resuspended in 4 mL of acetate buffer (pH 4.5) and gelatinised in a boiling water bath for 15 min. ST was hydrolysed by incubating the sample with 1 mL of amyloglucosidase solution (Sigma, 15 U mL^{-1}) at 50 ºC for 24 h. After cooling to room temperature, the samples were centrifuged (5000 *g*, 4 min). An aliquot (400 µL) of the supernatant was mixed with the anthrone reagent and absorbance at 639 nm was determined.

Glucose (GLU), fructose (FRU) and sucrose (SUC) were analysed enzymatically for carbohydrate concentration in samples of 60 to 70 mg, according to Steen and Larsson.[45] The extraction of soluble compounds from the plant powder was performed with 4 mL of 5 mM Hepes buffer (pH 7.0) at 50 ºC for 60 min.

Once the extraction suspension was precipitated at 1900 *g* for 10 min on a bench centrifuge, the upper fraction was 1:1 mixed with a polyamide suspension (80 mg mL^{-1}). The mixture was kept for 20 min with shaking after every 5 min, and centrifuged at 9000 *g* for 10 min.

In one part of the obtained solution, hydrolysis with 37 mM of H_2SO_4 at 80 ºC was applied. The double amount of GLU produced by hydrolysis defined the SUC concentration.

The GLU and FRU concentrations (including their hexose-6-phosphates) of different extracts were measured with the hexokinase and glucose-6-phosphate dehydrogenase indicator reaction, using the auxiliary isomerisation of hexose phosphates with phosphoglucose isomerase.[46-48] Changes in NADP absorbance at 340 nm were determined with a UV-VIS spectrometer Helios α (Unicam Ltd., Cambridge, UK). The enzymes were obtained from Megazyme (Bray, Ireland). Leaf carbohydrate concentrations were expressed in hexose mass units (mg g^{-1}).

Lignin (L) was determined as acid-insoluble (Klason) lignin.[49,50] The air-dried plant material was ground and extracted with acetone (100%) at

5 ºC, ethanol (96%), ethanol–benzene solution (1:1, v/v) and water at 60-70 ºC to remove the sugars, proteins, interfering phenolics and other soluble compounds. The residue was dried at 70 ºC for 24 h, and used for the determination of the acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent L (ADL).

For the determination of Fibertec fibres, the I&M Systems (Foss AB, Denmark) elaborated by Van Soest[49] were used. In the first step, NDF was determined after treatment with a neutral detergent solution (sodium lauryl sulphate and EDTA), the residue consisting of cellulose (CEL), hemicellulose (HC) and L.

The next step was to determine ADF after the treatment of residues with an acid detergent solution (cetyl trimethylammonium bromide, in sulphur acid solution). The residue contained CEL and L.

Finally, ADL was determined after the initial treatment for ADF measurement, followed by the removal of the CEL fraction through extraction with 72% H_2SO_4. A fraction of acid-soluble L and CEL could be lost during this procedure[49,50] The acid-resistant residue was recovered by filtration on a glass crucible with asbestos filter, carefully washed and dried at 70 ºC for 24 h to constant weight (Precisa 205A SCS, Switzerland). This residue contains only insoluble Klason lignin (hereafter called 'lignin').[50,51] After the residue was weighed, it was ashed at 525 ± 25 ºC for at least 5 h and N was calculated after correcting for mineral elements.[49]

Simple subtraction rules were used to calculate CEL: ADF – ADL = CEL. The results for L, HC and CEL were expressed as percentage of dry mass of plant material (% dw).

The analyses were performed in the Laboratory of Biochemistry of the Estonian University of Life Sciences.

Soil analyses

For interpreting the state of the trees and the relationships between wood quality and environmental conditions, the physical and chemical composition of the soil in the plantations was analysed while, for characterizing their quality, the ratios of elements in the soil were used to indicate the balance of nutrients for the trees. Taking into account that approximately 80% of the tree roots were actively engaged in the uptake of water, and that nutrients are located in the 0-30 cm layer from the surface,[52] soil samples were collected at this depth. The total N (%) in the soil samples was determined by the Kjeldahl procedure. To analyze the available P, K, Ca and Mg (mg kg^{-1}) present in the soil, the Mehlich 3 method was used. Soil pH in 1M KCl suspensions was measured in the 10 g: 25 mL ratio. For the characterization of the experimental plots, the arithmetic mean values of four analysed subsamples were used. The analyses were performed in the Laboratory of Agrochemistry of the Agricultural Research Centre and in the Laboratory of Plant Biochemistry of the Estonian University of Life Sciences.

Statistical analyses

To test the differences between the soil and tree growth parameters between plantations, *t*-tests were conducted, based on the assumption of unequal variances. For establishing the relationships between variables, Pearson's correlations – computed in MS Excel 2003 – were used. A significant effect was observed at a p-value < 0.05. The differences between plantations were tested with repeated measures of variance analysis (ANOVA), using Systat 10.0. The plantations (Kunda and Rapla) and tree compartments (foliage, branches and stems) were fixed factors, a variable for the compartments being the repeated measures on the trees nested in plantations. The differences in partitioning between the compartments were tested by the interaction term Compartment × Plantations.

RESULTS

Soil under plantations

The results of soil analysis showed significant differences between the studied plantations as to pH and nutrient concentration (Fig. 1). Although the soils had similar textures, being developed both on stony calcareous and on Ordovician limestone, the chemical composition of the soil in the Kunda plantation was significantly affected by the long-term dust pollution emitted from the cement plant. In these soils, the average pH was 0.5 units higher than that of the unpolluted plantation at Rapla. Differences in the properties of the upper layer of the soil were also evidenced in the level of nutrients. The concentrations of total and extractable Ca, K, Mg, P and N in the soil of the Kunda plantation were significantly different from those of the Rapla soil (Fig. 1).

The relatively high concentrations of Ca, Mg or K, registered in the upper layer of the soil, resulted in a nutritional imbalance for the trees. Consequently, the ratios of N/P, N/K and N/Mg were 2.3, 2.1 and 2.5 times lower in the Kunda plantation, whereas Ca/K was only 4% higher than in the Rapla plantation (Fig. 1).

Tree growth

After 12 and 13 years of growth, the mean H and DBH in the Kunda plantation were about 35% and, respectively, 42% lower than those for Rapla, while the *t*-test

revealed significant differences among the trees from the studied plantations (Fig. 2). The H and DBH in the plantations varied within relatively wide ranges: for Kunda – from 5.4 to 6.7 m and 3.9 to 4.9 cm, and for Rapla – from 6.8 to 10.3 m and from 5.5 to 9.6 cm, respectively. In 2010, no differences were recorded in the HI among the trees of our plantations. The DBH of trees in different plantations showed a strong correlation with HI (Kunda: r = 0.877, Rapla: r = 0.886). The tree growth was strongly correlated with the N accumulated in the leaves (Fig. 3), and with other inner factors, described below.

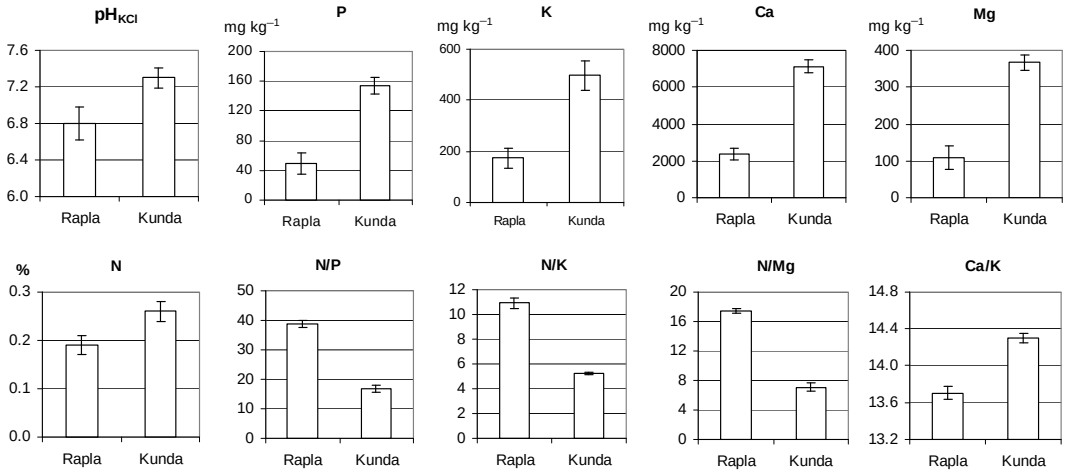

Figure 1: Average concentrations of nutrients and pH in soils of Rapla and Kunda plantations (n = 8-10). Differences (t-test) of soils between plantations and p-values for pH p = 0.000 (4.4E-0.5), for P p = 0.000; (6.91E-07); for K p = 0.0003 (1E-09), for Ca p = 0.007, for Mg p = 0.012 and for N p = 0.000 (6.46E-06)

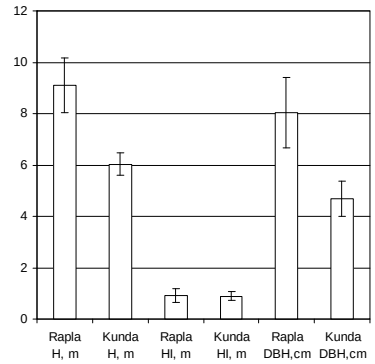

Figure 2: Average growth characteristics (± SD) of hybrid aspen in Rapla (12 yr. old) and Kunda (13 yr. old) plantations in 2010. Differences between trees in Kunda and Rapla plantations for height (H) were at p < 0.0011 significance, for height increment (HI) at p < 0.8146 and for diameter at breast height (DBH) at p < 0.0016

Carbon and nitrogen

The external supply of C and N may significantly affect plant growth and development, but little is known on the internal factors that influence development. Because leaves are more expensive to construct than other tree organs, the concentration of N in the leaves of aspen trees was much higher than in the branches and stems, varying significantly in trees from different plantations (Tables 1 and 2). Although C did not vary, there were differences in C partitioning in trees (Table 2). The study also showed strong differences in C/N among plantations and tree compartments (Tables 1 and 2). The correlations between N and C in different tree compartments and growth are given in

Figure 3. The nitrogen in the leaves was in a positive correlation with the H, DBH and HI of the trees from both plantations. Also, a positive correlation was established among N in the stems and the H and DBH of the trees. At the same time, C in the leaves correlated positively with the HI of the trees.

Non-structural carbohydrates

Hexoses and other water-soluble non-structural carbohydrates play a role in polysaccharide synthesis. Although the results obtained showed no differences in the C concentrations in the trees from different plantations, significant differences were observed in the concentrations of TSC and ST (Tables 1 and 2).

In the polluted Kunda area, TSC in aspen leaves was around 25%, on the average, in branches around 43% and in stems only around 9%, higher than the respective indicators at Rapla.

Table 1

Mean chemical characteristics (±SD) in different compartments of hybrid aspen trees and *p*-values of differences (*t*-test) among tree characteristics on Rapla and Kunda plantations, as to 2010 (n = 6-12)

Characteristics	Compartments	Rapla		Kunda	
			±SD		±SD
C, %	Leaves	46.26	0.76	46.74	0.38
	Branches	48.60	0.25	48.70	0.17
	Stems	48.10	0.58	48.20	0.49
N, %	Leaves	2.36	0.08	1.96	0.07
	Branches	0.57	0.07	0.44	0.03
	Stems	0.10	0.01	0.10	0.01
C/N	Leaves	19.60	0.61	23.85	1.05
	Branches	85.26	10.27	110.68	7.61
	Stems	466.14	32.87	509.67	50.12
TSC, %	Leaves	6.79	0.76	8.54	0.47
	Branches	3.54	0.52	5.07	0.43
	Stems	0.81	0.08	0.88	0.09
GLU, mg g^{-1}	Leaves	1.91	0.71	2.62	0.28
FRU, mg g^{-1}	Leaves	1.49	0.62	1.73	0.53
SUC, mg g^{-1}	Leaves	50.60	7.30	60.83	4.71
ST, %	Leaves	2.77	0.40	4.07	0.84
	Branches	1.33	0.14	2.77	0.24
	Stems	0.40	0.05	0.32	0.06
CEL, %	Leaves	16.06	0.65	14.50	0.64
	Branches	41.04	2.75	41.29	1.77
	Stems	61.65	2.51	61.34	1.18
L, %	Leaves	8.64	0.64	8.78	0.40
	Branches	18.91	1.63	15.47	0.76
	Stems	10.08	1.88	8.73	1.19
HC, %	Leaves	5.98	0.99	5.93	0.66
	Branches	13.20	0.58	15.82	1.98
	Stems	22.60	1.64	23.84	0.86
CEL/L	Leaves	1.87	0.12	1.62	0.05
	Branches	2.17	0.33	2.67	0.15
	Stems	3.44	0.86	20.81	0.37
CEL/HC	Leaves	2.75	0.49	2.44	0.37
	Branches	3.11	0.26	2.61	0.44
	Stems	2.72	0.21	2.57	0.16
L/HC	Leaves	1.44	0.12	1.49	0.14
	Branches	1.43	0.13	0.98	0.17
	Stems	0.45	0.06	0.36	0.02

Also, the ST concentration in the trees differed significantly, being higher in the leaves and in the branches by 45% and 108%, respectively, and lower in the stems by 20%, as compared to the results obtained for the Kunda area. According to ANOVA, the partitioning of TSC and ST between the tree organs was different in plantations (Table 2) while, in the stems, the concentrations of TSC and ST were less affected by the plantation setting (Table 1). In the concentrations of GLU and FRU in the leaves, no significant differences were observed, but differences in the transport sugar SUC were statistically significant, being 20% higher in the trees growing in the polluted area, compared to those growing at Rapla (Table 1). As to non-structural carbohydrates, relatively greater concentrations were registered in the leaves of Kunda trees.

Table 2
p-values of repeated ANOVA measures for the concentration of variables, by dry weight, and for their ratios as a function of plantation (Rapla and Kunda) and tree organ (foliage and branches)

Variable	Plantation	Organ	Organ × Plantation
C, %	0.132	0.000	0.366
N, %	0.000	0.000	0.003
C/N	0.001	0.000	0.008
TSC, %	0.000	0.000	0.611
ST, %	0.000	0.000	0.734
CEL, %	0.406	0.000	0.174
L, %	0.000	0.000	0.003
HC, %	0.023	0.000	0.022
CEL/L	0.110	0.000	0.002
CEL/HC	0.066	0.104	0.552
L/HC	0.013	0.017	0.043
GLU, mg g^{-1}	0.078		
FRU, mg g^{-1}	0.542		
SUC, mg g^{-1}	0.025		

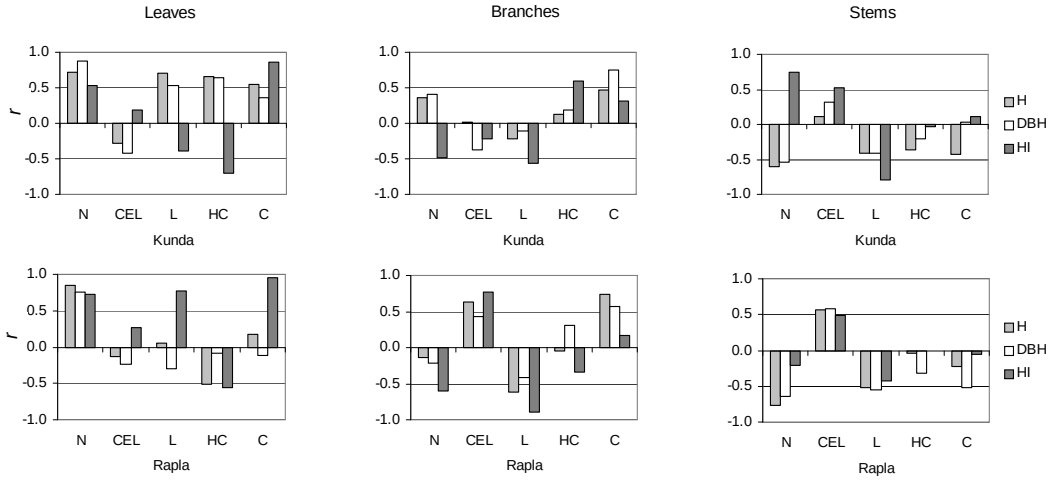

Figure 3: Correlation (at significance $p < 0.05$; r – correlation coefficient) between height (H), diameter of stem at breast height (DBH) and height increment in the current year (HI), and concentrations of N, C, cellulose (CEL), lignin (L) and hemicellulose (HC) in leaves, branches and stems of hybrid aspen in Rapla and Kunda plantations

Structural carbohydrates and lignin

The primary objective was to assess the dependence of structural carbohydrates, CEL and HC and L from hybrid aspen, on both quality of soils and intensity of tree growth. Substantial differences occur in CEL

partitioning in different tree compartments: stem> branch>leaf, while differences between plantations in CEL were found only for leaves (Table 1), the ANOVA test revealing a significant difference between tree compartments, as well (Table 2).

Repeated ANOVA measures indicated different responses in the L concentration of foliage, branches and stems between plantations. When comparing the sites, it was found out that, in Kunda, L concentration was smaller in branches and stems, while in leaves no significant differences were observed between sites (Tables 1 and 2). In branches and stems, HC was 20 and 6% higher in the Kunda plantation, but equal in leaves (Table 1).

The comparison of the trees from the plantations established in polluted and unpolluted areas revealed significant differences in the CEL/L ratio in all organs, while the difference in L/HC was statistically significant for plantations and compartments (Table 2). A negative correlation between the CEL and L contents was found for stems and branches in both Kunda and Rapla plantations (Rapla: $r_{branches} = –0.968$, $r_{stems} = –0.934$; Kunda: $r_{branches} = –0.505$, $r_{stems} = –0.786$).

As the initial substances for the synthesis of polysaccharides are non-structural hexoses, a positive correlation between CEL and FRU was found in foliage, both at Kunda ($r = 0.860$) and Rapla ($r = 0.790$).

Negative relationships were found between H, DBH and HI of trees and L in branches and stems, although a significant correlation was established only for HI. A weak but significant positive correlation between CEL for branches and stems was found. No significant trends were detected for the relationships between HC and growth parameters of the trees (Fig. 3).

DISCUSSION

Although hybrid aspen has been subjected to intensive selection and monitoring in experimental plantations in the hemiboreal region, few studies have focused on its growth under relatively extreme conditions, such as in industrial territories. Two plantations with hybrid aspens established on a former arable land in polluted and unpolluted areas showed great differences in soil pH and chemical composition, although the soils in plantations have been developed on a homogeneous parent material (stony calcarous till on Ordovician limestone).

In the plantation established close to the cement plant at Kunda, the long-term impact of dust pollution from the plant caused a significant rise in pH and in the contents of the predominant dust elements (Ca, K, Mg, P) in the soil. Taking into account[53,55] that the optimum soil pH for aspens is between 6 and 6.5(7), the soil in the Kunda plantation should be relatively unfavourable for aspens, comparatively with the Rapla one. Pinno *et al*.[31] evidenced that soil pH seems to be the major criterion to predict the productivity of hybrid poplar. Soil pH is one of the key factors in plant mineral uptake and mineral toxicity.[56]

According to DesRochers *et al*.,[53] the optimum soil pH for the growth of aspen is between 6 and 7. Thus, the pH of the soil in the Rapla plantation should be optimum for hybrid aspen. The results of the present work indicated that the growth of hybrid aspen on alkaline soil with a pH of 7.3 is possible; however, it is rather difficult and inhibited, as compared to that at a pH of 6.8 in the Rapla plantation. In the Kunda plantation, the H, DBH and HI of the trees were by 38, 42 and 4%, respectively, lower. Similar responses of hybrid aspens were evidenced by Timmer *et al*.[57] at pH 7.6, which reduced shoot dry weight by 33% while, at pH 6.6, the shoot reached maximum weight. At pH 8, soil only achieves a 60% germination, while values of 93 and 100% are achieved at pH 6 and 7, respectively.[58] Usually, a high pH may reduce the availability of both N, P and micronutrients.[59]

Carbon and N metabolisms are two major metabolic pathways, closely connected in almost every biochemical pathway, as well as in the growth, biomass and wood formation of trees.[20] Woody plants use both stored and currently produced carbohydrates, often simultaneously, for growth. Thus, the C status of plants affects developmental processes, being determined by the availability of N in the soil[59] and by its status in plants.[60] On former arable land in Southern Estonian plantations, the average foliar N is of 2.4-2.7%, described as sufficient for hybrid aspens.[16] In the Kunda plantation, foliar N was over 20% lower than that for unpolluted Rapla trees, and 25% lower than the optimum of 2.5% for hybrid aspen, as described by Jug *et al*.[61] The lower N in Kunda trees resulted in a significantly

higher C/N level, compared to that of Rapla trees. As, generally, the C/N ratio is important for tree development, but especially for root formation,[62,63] the increase in C/N in the Kunda plantation would support the hypothesis of van den Driessche,[64] according to which a reduced N supply may restrict root growth and thus vegetative growth of trees. The C/N ratio of plants can be increased even with a minimal decrease in the growth rate.[65] These findings suggest that, although no differences occurred in the total C concentration between trees from different plantations and in C partitioning between the tree organs, an altered partitioning of starch and of other non-structural carbohydrates was established.

Before discussing the biosynthesis of different polysaccharides and lignin in the plant cell walls, a description of the key substances involved in the process is needed. Although the accumulation of non-structural carbohydrates in tree leaves is explained by both photosynthesis and foliage N concentration,[20] no positive correlations were found between the contents of N and the non-structural carbohydrates in the trees. In the trees from the Kunda plantation, foliar TSC, hexoses and SUC were higher than those obtained under the optimal pH at Rapla. However, earlier studies of Mandre[66] showed that, under alkaline dust pollution, at soil pH values of about 7.7-8.1, TSC in Norway spruce needles decreases during the whole season. Clinker dust and wood ash application appears to decrease the FRU and GLU concentrations in needles, while the SUC concentration was less affected by the modified nutrition.[67] Of course, the responses of trees to environmental factors may be species-specific and there might be different responses to the alkalisation of soils. Anyway, today it is difficult to explain the increased level of TSC, GLU, FRU and SUC in the leaves of aspen on a territory of high pH and K, Ca and Mg concentrations in the soil. The TSC differences in leaves and branches between the plantations observed in the present study were in accordance with the osmotic salt stress and imbalance in tree mineral nutrition at Kunda, where the soil had excess of base cations, elevated pH and possibly reduced availability of N for trees. The possibility of N shortage was assumed by Liu *et al*.,[68] who showed that composites Mg, K and Ca fertilisation and pH increase in the soil magnifies starch reserves in sugar maple leaves.

Also, salt treatments, which osmotically inhibit the water uptake by roots, increase SUC, mannitol and raffinose levels, while the concentrations of GLU and FRU differ less in aspen.[69] Relatively small differences in foliar GLU and FRU between plantations, and larger SUC and TSC concentrations at Kunda may indicate the effect of the relatively high concentrations of mineral elements in the soil on the foliar soluble carbohydrates present. Drought and osmotic stress commonly cause increased hydrolysis of ST, to generate soluble carbohydrates when photosynthesis is restricted, due to low stomatal conductance during stress.[20] In contrast to the study of carbohydrates in oak leaves, where the ST level was reduced in response to the osmotic stress caused by water shortage,[70] at Kunda, foliar ST concentration was higher than that of the Rapla plantation.

In the present study, higher SUC levels in aspen leaves were found in the polluted Kunda plantation. The positive correlation between GLU and SUC in leaves is rational, because GLU is converted – through the metabolic pathway – to ST or to other sugars, such as glucose-6-phosphate or fructose-6-phosphate and then to SUC.[17] Also, in the needles of *Picea abies* and *P. glauca*, an increase of SUC was established on the alkalised territory in the vicinity of the cement plant.[71] In another experiment, Klõšeiko and Tilk[72] evidenced the stability of the SUC concentration in *Pinus sylvestris* needles after soil treatment with clinker dust. As SUC is transported – as sap – to the processing centres of the cambial region of the main stem, branches and roots, SUC plays an essential role in wood formation. Konishi *et al*.[22] indicated that the SUC imported from the phloem can be either directly converted to CEL or to other cell wall carbohydrates through the activity of SUC synthase.

In the present study, the lower N concentration and smaller height of trees at Kunda could explain the increased ST levels in leaves and branches. The shortage of N increases ST concentration in leaves.[73] The interrelationships between foliar ST and N concentration under N limited conditions is well known.[74,75] When N is limited, it has a more dominant role than the photosynthetic

capacity in affecting plant growth and development.[75] Along with a reduced possibility for building up N reserves, such as proteins and aminoacids, more C remains available for ST synthesis in N deficient trees.[73,74] Nitrogen shortage reduces growth, while maintaining photosynthesis as a priority. As a consequence, the ST in foliage accumulates, due to reduced sink activity. The ST reserves in the above-ground parts are not depleted for root growth and mineral uptake under deficitary conditions. Such partitioning could be regulated by phytohormones, such as abscisic acid and cytokinins, involved in the control of metabolic activity in the given plant part, and in biomass partitioning in herbaceous plants.[76,77] However, in the present study, the current growth, measured by the length of the upper stem node, was not significantly different between sites. Height increment could be smaller in taller trees and their assimilates are possibly allocated to a greater extent to wood formation in supportive parts, such as branches and stems.

The external increase in the pH and Ca and K levels may stimulate lignification of trees. Earlier positive correlations were found between the L content of Scots pine needles and shoots, while K and Ca in the soil were influenced by cement dust.[78] For the hybrid aspen clones from four plantations in the Southern regions of Estonia, no correlation was established[15] between stem L concentration and pH of the soils.

In the branches and stems of the trees growing at higher pH and Ca, K, Mg concentrations in the Kunda soil, L was lower than at a relatively optimal soil pH, while the CEL concentrations in branches and stems did not vary between the two plantations. Negative correlations were found between CEL and L in branches and stems for the trees growing in the studied plantations, as found earlier in Southern Estonian plantations for stem wood.[15] The negative correlations may be thought of as a competition for C allocation to L *versus* C allocation to CEL and HC, as these two classes of molecules are the major C sinks in the formation of the wood cell wall. The strength and direction of the transport of carbohydrates and C partitioning in trees have been demonstrated to be regulated by source–sink relationships, which may be also varied by external factors.[17,79,80] Hu *et al*.[36] indicated that L and CEL deposition could be regulated in a compensatory fashion, which may contribute to metabolic flexibility and a growth advantage to sustain the long-term structural integrity of trees. Although ascribing the phenomenon to a discrete causal mechanism is not yet warranted, several points of discussion can be offered. An increased CEL/L ratio at a lowered L concentration in the branches and stems of hybrid aspen from the Kunda plantation may be a compensatory regulation of CEL and L deposition, and could represent a tree-specific adaptation to sustain mechanical strength in lignin-lowered trees. This interrelated phenomenon was described for most severely lignin-reduced transgenic *Populus* trees.[36] Unlike the Rapla plantation, at Kunda, the correlation between L and HC in branches was significant (r = –0.901). However, the quantities of cell wall HC in branches were increased by about 20%, with a reduction in L in the Kunda plantation, compared to the Rapla one.

Cellulose is a linear chain of ß (1-4) linked D-glucose units, but HC is derived from several sugars in addition to GLU, xylose etc.[81] This means that the soluble carbohydrate contents in trees become important for CEL and HC synthesis and, finally, for wood formation.[82] A comparative analysis of trees in the Kunda and Rapla plantations showed that the leaves from the unpolluted plantation had significantly higher concentrations of CEL, while the CEL in stems and branches did not vary between plantations. HC concentration was higher in the polluted plantation at Kunda, especially in branches. A different partitioning of CEL and HC between different compartments of trees may be caused by the activity of enzymes participating to the synthesis and accumulation of these polysaccharides.

Lignin is the third major component of wood, whose deposition is preceded by carbohydrate deposition, being influenced by the chemical nature of the cell wall carbohydrates and orientation of CEL microfibrils.[12] A negative correlation of biomass growth and L content was shown for 396 genotypes of the *Populus* family,[82] as supported by the results obtained for hybrid aspens. It is known that, within a plant, the L content can vary greatly in different tissues, and may have a great deal of variation in its chemical composition and physical properties.[18] This may be an explanation for the great differences in L concentrations

between organs and plantations, as revealed by the ANOVA study.

CONCLUSIONS

The results obtained on the cell wall components and soluble carbohydrates of hybrid aspen on former agricultural lands showed the dependence of growth and wood formation on the nutritional level and soil pH. The high level of the soil pH and Ca, K and Mg resulted in a significant increase of both C/N and CEL/L in the trees. The changes in the ratios of CEL, HC and L indicated a possible changing in wood quality, by the regulation of mineral nutrition and TSC content of the trees. The significant increase of non-structural carbohydrates, including SUC, in the leaves of the trees from the polluted plantation may indicate rearrangements in the translocation processes of carbohydrates.

It is difficult to interpret the causal relationships between carbohydrate metabolism and wood quality under the described complex of experimental conditions. Still, it is possible to state that the physiological state of hybrid aspen is rather sensitive, as expressed by the growth of hybrid aspen in the plantations under study.

ACKNOWLEDGEMENTS: The study was supported by the Estonian Ministry of Education and Research (project No. 0170021s08) and the Estonian Science Foundation (grant No. 7298).

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