Drought tolerance of Scots pine in diverse growth conditions on a dune estimated on the basis of carbohydrates and chlorophyll fluorescence in needles

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Abstract. Variation in the needle carbohydrate concentrations in Scots pine along the height profile on a forested dune in south-western Estonia were measured in spring. To assess the tolerance of trees, carbohydrate concentrations were compared with shoot morphology and photosystem II quantum efficiency (chlorophyll fluorescence). To test a potential effect of drought events on trees, chlorophyll fluorescence was measured in needles after drying and recovery, and dependencies of the treatment effects on the location on the dune were looked for. Water concentration in detached needles was reduced by a partial drying in laboratory. Needles were recovered by an addition of water. Carbohydrate concentrations were rather constant irrespective of shoot morphology and the tree location on the dune. The reduction in the photosynthetic efficiency, observed after the treatment, depended on the site. The results indicated that trees growing at lowest relative heights on the dune were potentially more vulnerable to drought than other trees on the dune at relative height of 5 to 25 m.

Key words: drought tolerance, environmental gradient, quantum efficiency.

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Introduction

Scots pine stands dominate on dunes in the coastal area of south-western Estonia (Pärn, 2003). On the top of dunes sparse stands have developed, while at the lower elevations the stands are denser and productive. The soil nutrient availability varies largely depending on the elevation (Mandre, 2003). Soil water content has not been investigated, but differences in the water availability modified by season and vegetation are likely (Ensign *et al.*, 2006). At higher locations, periodic drought events may occur due to soil hydrological properties, wind and less shading by trees.

Nutrients and water in soil can significantly affect carbohydrate concentrations in plants. Generally mineral deficiencies impair carbohydrate consumption, which results in accumulation of carbohydrates in foliage (Ericsson *et al.*, 1996; Ludovici *et al.*, 2002). Water deficit usually causes accumulation of soluble carbohydrates in foliage to maintain the water content in leaves with the help of osmotic mechanisms. On a dune the soil is poorer in nitrogen on the top than on the foot (Mandre, 2003).

Observational studies of forests on environmental gradients are used to develop hypotheses about mechanisms of trophic interactions and adaptation in forest ecosystems (Högberg *et al.*, 2003; Niinemets & Lukjanova, 2003). Whether there are significant differences in the carbohydrate concentrations of trees on a natural environmental gradient is not clear. A previous study conducted on a dune in autumn indicated large differences in light interception by stand and modest differences in the carbohydrate concentrations in the needles of Scots pine, except perhaps for sucrose (Klõšeiko, 2003). Differences in soil water and nutrients suggest corresponding differences in tree carbohydrates at least at some point of the seasonal development of trees. Alternatively, based on microbial respiration, biomass and composition, Högberg *et al.* (2003) propose a hypothesis that nitrogen exchange from soil with carbon from trees reaches a balance so that basic physiological processes in soil are not much affected by the variability in abiotic factors. This could also indicate stabilized carbohydrate metabolism in trees, particularly so because on top of the dunes where stands are sparser, light availability for photosynthesis and thus for carbohydrate production is better than at lower elevations. Excess light could cause also stress during winter when the trees on the top of the dune are influenced by desiccation.

Previous measurements were conducted in autumn when starch is virtually absent from needles. In contrast, spring is the season when starch accumulates extensively in conifers. Because starch functions as a buffer for carbohydrate production and consumption its concentration is readily affected by environmental conditions. Trees could suffer from the winter stress or be rather well acclimatized to drought stress also in spring. In some studies higher tolerance to winter conditions is associated with improved tolerance to drought (Blödner *et al.*, 2005). Stress could also damage and weaken the trees.

Photosystem II quantum efficiency (transfer of exciton energy to photosynthesis) measured by chlorophyll fluorescence is used in ecophysiological studies to measure the performance or vulnerability of plants. The lifetime of exciton is extremely short. However, energy cannot disappear and when the exciton has no possibility of migrating, the energy is lost in the form of heat or chlorophyll fluorescence. For successful migration between molecules, the components of photosynthetic membranes must be specifically aggregated. One reason for the dissipation of the absorbed light energy in the form of fluorescence could be a structural disorganisation in photosynthetic membranes. Abiotic factors tend to disorganise cellular structures. It was suggested that in aspen leaves, carbohydrates help to maintain the structural integrity of photosynthetic membranes during heat stress as addition of carbohydrate solutions increases the temperature of fluorescence rise (Hüve *et al.*, 2006). In drought tolerant Norway spruce seedlings the photosynthetic quantum efficiency decreases several days later than in less tolerant seedlings in the progression of drought treatments (Blödner *et al.*, 2005).

The aims of the current study were (1) to find whether the concentrations of hexoses (fructose and glucose), sucrose and starch in Scots pine needles vary in different locations on dune in spring, (2) to detect relationships of carbohydrate concentrations with shoot morphology and chlorophyll fluorescence, and (3) to evaluate whether the effect of needle drying and recovery on chlorophyll fluorescence depends on the location on the dune to measure the tolerance of trees and potential effect of drought events.

Material and Methods

Sample collection

There had been no rain for three weeks, and the Estonian meteorological service issued the highest forest fire alert. At the beginning of May 2006, on the day of sample collection the sky was clear and the air temperature was 18 °C. The forest floor was completely dry. At each location of the 230 m long west-east transect on the dune a single branch was cut from 9 trees within the 20 to 40 m long band at the same elevation around midday. Twenty current-year needles from each branch were immediately put in transparent plastic bags (8 × 6 cm, 40 µm wall thickness) and sealed for fluorescence measurements made later. Sample collection lasted 2 h. The shoots were kept in larger plastic bags to reduce loss of water during transportation.

Needle and shoot morphometry

Current-year shoots were dried at 60 °C for 48 h. Three shoots from a tree were measured for length and dry mass. The same shoots were used to measure 5 samples of 2 needle pairs. The ratio of mass per unit length (mg cm⁻¹) of shoots and needles was calculated. As shade needles of Scots pine tend to be relatively thin (Niinemets & Lukjanova, 2003), the mass to length ratio of needles could be an indicator of light conditions on the sampling location and in the tree crown.

Carbohydrate analysis

Current-year needles from three trees were joined and fixed (3 to 4 g of needles) by microwave heating on filter paper with 3 ml of ethanol in beaker in microwave oven at 800 W for 2 min. Three samples were fixed simultaneously in the oven. The needles were completely dried at 60 °C for 48 h. Drying was followed by grinding in a vibrating ball mill MM200 (Retsch, Haan, Germany). Carbohydrates were extracted from an about 50 mg sample in 5 ml of distilled water at 40 °C for 1 h. The extracts were cleared on a bench centrifuge at 1900 g.

Phenolic compounds could inhibit the activity of enzymes due to strong hydrogen bonds between proteins and polyphenols. Some phenolic compounds oxidize easily by the dissolved oxygen, particularly at alkaline pH. Enzymatic reactions were conducted at pH 7.6. Quinone-protein adducts could be formed. Phenolics absorb in the UV range, which is also used for the carbohydrate determination (at 340 nm). The background absorbance could change when phenolics oxidize during the spectrometric determinations. To reduce the concentration of phenolics (Donner *et al.*, 1993), the clear extracts were poured to tubes containing ~100 mg insoluble polyvinylpyrrolidone (cross linked, AppliChem, Darmstadt, Germany), mixed during 30 min, and centrifuged.

From the clear and purified solutions glucose, fructose and sucrose were analysed enzymatically (Steen & Larsson, 1986). According to the D-fructose and D-glucose assay procedure (2005), 0.4 mg ml⁻¹ of soluble polyvinylpyrrolidone PVP40T (Sigma-Aldrich, Steinheim, Germany) was included in the mixture of enzymatic reaction. The enzymatic method has good selectivity, which permits analysing the extracts with little or no purification.

We tested the removal of phenolics by polyamide according to Collins *et al.* (1998). With soluble polyvinylpyrrolidone in the reaction mixture, the test analyses from purified and unpurified extracts were about equal (Table 1). Fructose readings tended to be smaller and the background absorbance was significantly higher with-

Table 1. Effect of solid-phase clean-up of extracts with polyamide on the determination of carbohydrates. Current year pine needles in september were analysed. Carbohydrates parameters are expressed in mg g⁻¹ needle dry mass. Background = absorbance of the reaction mixture at 340 nm before the addition of enzymes. *p*-value = result of Wilcoxon signed rank test for pair-wise differences (*n* = 7).

Tabel 1. Polüamiidiga puhastamise mõju süsivesikute analüüsile männi okaste ekstraktidest. Süsivesikute sisaldus on esitatud mg g⁻¹ kuivmassi kohta. Taust = reaktsioonisegu neeldumine 340 nm juures enne ensüümide lisamist. *p*-väärtus arvutati Wilcoxon'i mitteparameetrilise paari-kaupa võrdlemise testiga (*n* = 7).

Parameter / Näitaja	Raw extract Puhastamata ekstrakt	Purified extract Puhastatud ekstrakt	<i>p</i> -value
Glucose / Glükoos	2.63	2.65	0.87
Fructose / Fruktoos	2.89	3.04	0.06
Glucose+Fructose	5.52	5.69	0.40
Sucrose / Sahharoos	39.7	39.8	0.87
Fructose/Glucose	1.07	1.12	0.09
Sucrose/(Glucose+Fructose)	7.74	7.55	0.50
Background / Taust	0.23	0.02	0.02

out the solid-phase cleanup. Current background for the dune samples was 0.08 ± 0.05 (*n* = 24), which is larger value than obtained with the test analyses (Table 1). Therefore some phenolics from pine needles possibly remained in the solution. For example, flavonoid glucosides bind weakly to polyvinylpyrrolidone (Donner *et al.*, 1993). However, as the test with completely unpurified extracts indicated only small differences, the results for dune were probably not affected by the incomplete removal of interfering substances from the extracts.

Starch was analysed from the insoluble residue with thermostable α -amylase (162 U/ml; A-4551; Sigma-Aldrich, Steinheim, Germany) and amyloglucosidase (235 U/ml; from *Aspergillus niger*; Merck, Darmstadt, Germany) almost in line with the AACC Method 76.13 (Total starch assay procedure, 1998). Starch was solubilised with dimethyl sulphoxide (1.5 ml) and α -amylase using microwave heating (2×12 tubes in microwave resistant racks at 150 to 240 W). The digestion volumes were reduced to 4 and 0.3 ml for α -amylase and amyloglycosidase treatments, respectively. Carbohydrate concentrations were expressed in mg hexose units g⁻¹ needle dry mass.

Fluorescence measurements

Chlorophyll fluorescence of the needle samples (needles in transparent plastic bags) was measured 3–4 h from the collection in the field, after the needles were dried for 3 days, and 2–8 days after rewatering. Needle samples were adapted at low light ($4\text{--}10 \mu\text{mol m}^{-2} \text{s}^{-1}$) under daylight lamps at 22 °C for 30 min. Fluorescence was measured through the bag wall from the light adapted side with a chlorophyll fluorometer PPM200 (EARS, Delft, The Netherlands). Photosystem II quantum efficiency (Genty parameter, %) is the relative increase of fluorescence intensity upon the saturating light pulse over the ambient fluorescence. The efficiency is for primary mechanisms independent of the measurement area. Fluorescence measurements can be used to screen repeatedly a large number of samples in a short time.

During initial measurements the mass of the samples was recorded. To reduce the water content of needles, the bags with needles were kept equally open with the

help of a v-shaped filter paper insert for 3 days in laboratory away from direct sunlight. After drying the sample mass was recorded to find the mass loss (% of the initial mass) during the drying. In the calculations, the bag mass was subtracted. On average the mass of the needles was reduced by 0.17 ± 0.05 g ($n = 72$). An about 5×0.5 cm filter paper strip was put on the bottom of the bags for uniform water adsorption near the needle bases, 0.25 ml of distilled water was added, and bags were closed. The samples were further kept on a vertical frame at low daytime light. Chlorophyll fluorescence measurements were continued 2, 4 and 8 days after the addition of water.

Statistical analysis

Averages per tree and carbohydrate sample were calculated. Data were transformed to meet the normality requirement for tests using skewness and kurtosis in basic statistics. Mass and mass to length ratio of shoots and needles, individual shoot length, sucrose to hexoses ratio, sample mass in bags and loss of mass during drying were transformed logarithmically because of their positive skewness. Combination of subtraction from constant, logarithmic and square transformation was used for the chlorophyll fluorescence measured after recovery. This parameter had negative skewness. Density plots, summary charts and scatterplots were drawn. Analysis of variance and covariance in the general linear models in Systat 10.0 for Windows (Systat Software GmbH, Erkrath) was used to test differences between sample locations and statistical influence of the relationships between the parameters on the differences. The strength of association was presented by multiple correlation coefficient (r). Significance of tests was accepted at probability level $p < 0.05$. The Kruskal-Wallis test and exclusion of a data point were used to check the probabilities when the software indicated an exceptionally different value.

Results

Morphometric characteristics of needles and shoots

Analysis of variance revealed significant differences between the transect locations only in mass and mass per needle length ($p < 0.001$ – 0.01). Significant effects on mass and nonsignificant effects on length were observed also for shoots with about the same statistics as for needles (Table 2). The heaviest needles and shoots with a high mass to length ratio were on the top of the dune. On the bottom of the east side trees had quite large needles as well. The mean mass and shoot parameters of a branch varied considerably between the trees.

Carbohydrates

The lowest hexose concentration in needles was observed on the east slope of the dune (Figure 1). Significant differences in glucose, fructose and their sum were detected by excluding one exceptional data point ($p < 0.05$). The Kruskal-Wallis tests for glucose, fructose and their sum did not show significant differences ($p = 0.07$ – 0.18). However, significant differences were found for sucrose (Kruskal-Wallis test, $p < 0.05$). Sucrose varied less between the trees in a sampling band on the transect than hexoses relative to the mean value in the band. The starch level was the highest on the top of the dune ($p < 0.05$); the other locations did not show any marked differences. The ratio of fructose to glucose on the transect did not vary significantly. The sucrose to hexose ratios were larger on the west slope and in the eastern marginal location (with one exceptional data point excluded $p < 0.01$, p -value by Kruskal-Wallis test = 0.09).

Table 2. Morphometric characteristics of Scots pine needles and shoots on a dune in May. Sampling locations from west to east (6 = top). Mean $\pm$ SD, $n = 9$ trees.

Tabel 2. Hariliku männi okaste ja võrsete morfoomeetrilised näitajad kevadel luitel. Proovikogumise kohad on tähistatud läänest itta (6 = tipp). Keskmine $\pm$ SD, $n = 9$ puud.

Organ / Organ	Sampling location Koht	Length, cm Pikkus, cm	Dry mass, mg Kuivmass, mg	Mass/Length, mg cm ⁻¹ Mass/Pikkus, mg cm ⁻¹
Needles / Okkad	1	5.9 $\pm$ 1.1	7.8 $\pm$ 2.1	1.3 $\pm$ 0.3
	2	5.5 $\pm$ 0.5	6.6 $\pm$ 1.2	1.2 $\pm$ 0.2
	3	5.1 $\pm$ 0.9	6.8 $\pm$ 2.1	1.3 $\pm$ 0.2
	4	4.3 $\pm$ 0.6	5.8 $\pm$ 1.4	1.3 $\pm$ 0.2
	5	4.9 $\pm$ 0.8	8.0 $\pm$ 2.9	1.6 $\pm$ 0.3
	6	5.6 $\pm$ 0.8	12.0 $\pm$ 2.5	2.1 $\pm$ 0.3
	7	5.2 $\pm$ 0.5	6.9 $\pm$ 1.2	1.3 $\pm$ 0.2
	8	6.0 $\pm$ 0.9	10.2 $\pm$ 3.8	1.7 $\pm$ 0.4
Shoots / Võrsed	1	6.2 $\pm$ 3.6	218 $\pm$ 174	31 $\pm$ 11
	2	3.3 $\pm$ 1.2	99 $\pm$ 67	27 $\pm$ 8
	3	4.7 $\pm$ 1.8	174 $\pm$ 126	33 $\pm$ 10
	4	3.5 $\pm$ 0.7	97 $\pm$ 36	26 $\pm$ 5
	5	5.4 $\pm$ 2.0	230 $\pm$ 156	38 $\pm$ 16
	6	6.4 $\pm$ 2.8	470 $\pm$ 403	63 $\pm$ 22
	7	5.4 $\pm$ 2.0	197 $\pm$ 148	30 $\pm$ 11
	8	6.3 $\pm$ 3.1	332 $\pm$ 311	45 $\pm$ 16

Figure 1. Concentration of carbohydrates in Scots pine current-year needles on a dune at different locations on a transect from west to east (point 6 = top) in May. Mean $\pm$ SD, $n = 3$ samples joined from 9 separate trees.

Joonis 1. Süsivesikute sisaldus jooksva aasta okastes maiuus harilikul männil lääne-ida suunalisel transektil luitel (punkt 6 = tipp). Keskmine $\pm$ SD, $n = 3$ ühendatud proovi 9 puult.

Partly the variance in hexoses could be caused by local differences in the canopy and crown positions where needle and shoot morphology differ. Therefore it was tested whether variance in carbohydrates was explained by the variance in needle and shoot morphology between the trees. Mass and mass to length ratio were highly correlated between shoots and needles ($r = 0.89$ and 0.9 , $p < 10^{-7}$, $n = 24$). Accordingly, relationships presented below for needles were valid also for shoots between these mass related parameters and carbohydrates. Sucrose, starch and the ratio of fructose to glucose were statistically dependent on needle morphometric characteristics. The sucrose concentration was larger in heavier needles and in the case of higher mass per length ($r = 0.54$ – 0.6 , $p < 0.01$). Shoot length was positively correlated with sucrose concentration ($r = 0.44$, $p < 0.05$). Also the starch concentration was larger in heavier needles and in the case of higher mass per length ($r = 0.47$ – 0.5 , $p < 0.05$). Shoot length was positively correlated with starch concentration ($r = 0.44$, $p < 0.05$). When one exceptional data pair was excluded sucrose and starch were significantly correlated ($r = 0.57$, $p < 0.01$). The fructose to glucose ratio was smaller in shoots with higher mass. In needles the relationship between the fructose to glucose ratio and mass was close to significant. The negative relationship of the fructose to glucose ratio with morphology was in accordance with the positive relationship of starch with morphology and the correlation between starch and glucose ($r = 0.48$, $p < 0.05$, $n = 23$).

Chlorophyll fluorescence

Within the initial measurements before additional treatment in laboratory, the largest difference between the average chlorophyll fluorescence was 3.6% in the Genty parameter units, which was found between the top and the west slope ($p < 0.01$) (Table 3). The sample mass put in bags was a marginally significant covariate (p -value = 0.067). Drying until about 20% mass loss from the initial mass of the separated needles resulted in marked reduction of the Genty parameter (to about 40%). Differences between sampling locations increased and so did variance. Drying reduced the effi-

Table 3. Photosynthetic efficiency by chlorophyll fluorescence (Genty parameter, %) of detached Scots pine needles collected from a dune from west to east (6 = top). Needles were measured 3–4 h after collection (initial Genty parameter), after drying for 3 days, and after 4 days of recovery with water. Mass loss = decrease in sample mass due to drying. Mean of 9 trees, $\pm$ SD.

Tabel 3. Eraldatud okaste fotosünteesi kasutegur klorofüllü fluorestsentsi järgi (Genty parameeter, %) luite erinevatel kohtadel läänest itta (6 = tipp). Fluorestsentsi mõõdeti 3–4 tundi pärast kogumist (algne Genty parameeter), pärast kuivatamist 3 ööpäeva ja pärast okaste taastumist veega. Massi kadu = okkaproovide massi vähenemine kuivatamise lõpuks. Keskmine $\pm$ SD 9 puult.

Location Koht	Initial Genty parameter / Algne Genty parameeter	After drying Pärast kuivatamist	After recovery Pärast taastumist	Sample mass, g Proovi mass, g	Mass loss, % Massi kadu, %
1	69.7 $\pm$ 1.9	33.1 $\pm$ 5.6	55.1 $\pm$ 7.5	0.8 $\pm$ 0.2	22 $\pm$ 6
2	68.7 $\pm$ 1.9	39.6 $\pm$ 7.5	57.2 $\pm$ 5.6	0.6 $\pm$ 0.2	22 $\pm$ 3
3	71.0 $\pm$ 1.4	44.0 $\pm$ 9.5	61.0 $\pm$ 3.2	0.9 $\pm$ 0.2	20 $\pm$ 4
4	71.3 $\pm$ 1.5	38.2 $\pm$ 7.2	61.8 $\pm$ 3.3	0.7 $\pm$ 0.2	22 $\pm$ 3
5	71.6 $\pm$ 1.7	44.1 $\pm$ 6.4	61.9 $\pm$ 3.1	0.9 $\pm$ 0.4	19 $\pm$ 2
6	72.3 $\pm$ 1.5	41.9 $\pm$ 5.2	56.2 $\pm$ 5.8	1.2 $\pm$ 0.2	19 $\pm$ 2
7	70.4 $\pm$ 1.5	42.9 $\pm$ 5.3	60.8 $\pm$ 7.6	0.8 $\pm$ 0.2	20 $\pm$ 3
8	70.2 $\pm$ 1.3	39.2 $\pm$ 6.3	54.2 $\pm$ 4.6	1.2 $\pm$ 0.5	20 $\pm$ 4

ciency of the exciton transfer more in the needle samples collected from the lower parts of the dune than from the top (analysis of variance for differences from the initial measurements was significant at $p < 0.05$). The effect of the location on the change in the Genty parameter during drying was also significant ($p < 0.001$) when the initial needle mass was included in the model. Loss of mass during drying depended on the initial sample mass. Smaller samples lost more mass relative to their initial mass. Reduction in the Genty parameter was correlated with the loss of sample mass ($r = 0.44$, $p < 0.001$, $n = 71$).

After drying the needles were recovered by the addition of water. Little recovery was observed after two days with water (the average Genty parameter increased from 40% to 43%). The best recovery was found after 4 days (Table 3). On the 8th day the Genty parameter was 55% on average and the standard deviations had increased. Therefore the data after 4 days from the water addition were used for further analysis of recovery. The increase in the Genty parameter after the rewatering of the needles was not significantly different between the transect locations (p -value = 0.117). Differences between the locations were even less significant when sample mass or mass loss was used as a covariate. Recovery was larger in the samples with larger mass loss during drying ($r = 0.38$, $p < 0.001$, $n = 72$). In the recovered samples, no statistical dependence of the Genty parameter on the initial sample mass and mass loss was found.

Regression of the Genty parameter on the sum of hexose, fructose, sucrose, starch, ratio of fructose to glucose and sucrose to hexoses was tested with and without the mass of the samples used for the fluorescence measurements. The initial Genty parameter was larger in needles with higher hexose and fructose concentrations and accordingly larger in the needles with a smaller ratio of sucrose to hexoses ($r = 0.59$ – 0.65 , $p < 0.01$ – 0.05 , $n = 23$). When the transect location was included in the model, the remaining partial regressions were nonsignificant. No dependence was found for the decrease in the Genty parameter due to drying with the carbohydrate concentrations. After recovery the photosynthetic efficiency tended to be smaller in the needles with larger starch and sucrose concentrations ($r \sim 0.49$, $p < 0.05$, $n = 24$).

Discussion

Statistically significant variation was observed in the carbohydrate concentration of the needles from the pines growing on the transect along diverse conditions on the dune. However, the differences were not clearly related to the height profile. The soils of the top and lower parts of the dune differ substantially in nutrient availability (Mandre, 2003). Towards the top the N and K concentrations in soil are low. In needles there is less variability in the mineral nutrients, although deficiency of N and K in tissues could be regarded as limiting factor (Mandre, 2003). It is well known that nitrogen and potassium deficiencies have often a marked influence on the carbohydrate metabolism and concentration in trees. Possibly needles were adapted to the low availability of nutrients in soil before the new growth in spring. On the top of the dune the crowns of pines were well exposed to light from every side. This might explain the higher starch accumulation and larger mass and thickness of needles on the top. Despite a period of dry weather at the time of sampling, the water availability from soil was probably not a limiting factor because of small evaporation in the conditions of cool temperatures and soil water reserves gathered during winter and early spring accessible by the extensive root system of pine.

The effect of dune location on hexoses and sucrose was of interest. In previous studies, to overcome of K deficiency manipulation of soil nutrients by wood ash and clinker dust treatment caused a decrease in hexoses while the sucrose concentration was rather stable (Klõšeiko & Tilk, 2006; Klõšeiko, 2006). The ratio of hexoses and sucrose depends on the sink activity and may indicate or regulate the balance between the source and sink in herbaceous plants (Moore *et al.*, 1999; Rogers *et al.*, 2004). Experimental reduction of the carbohydrate consumption by leaf girdling resulted in a larger increase in hexoses relative to sucrose in the starch storing vegetable crops (Goldschmidt & Huber, 1992). On the top of the dune, good light conditions may favour carbohydrate production. The carbohydrates allocated to roots may further facilitate the uptake of mineral nutrients by providing a resource for the heterotrophic soil processes, which are essential for the uptake of the low concentration nutrients from soil to trees. In the present study, the hexose concentration and its ratio to sucrose matched best the height profile of the dune. The smaller ratio of sucrose to hexoses on the top of the dune is in accordance with the nutrient poor soil and results of the studies on the manipulation of soil nutrients with wood ash and clinker dust. However, the relatively large shoots and needles are contradictory to the mineral deficiency on the top of the dune during the previous growth period. As splitting of sucrose to fructose and glucose increases the number of solute particles without changing the carbon balance, the partitioning between sucrose and hexoses could be also a flexible way to adjust osmotically. Higher hexose concentration is in accordance with the osmotic adjustment to the assumed dryer conditions at the higher elevations. However, the hexose concentration was low and therefore it is reasonable to assume that hexoses did not substantially affect the osmotic pressure in needle cells. Thus the smaller ratio of sucrose to hexoses could indicate that on the top of the dune the production of carbohydrates was relatively active compared to their consumption for starch accumulation and allocation to roots. Somewhat exceptional carbohydrate concentrations on the west foot might be due to the characteristics of the stand and the relative position of crown accessible for sample collection.

The efficiency of primary exciton transfer mechanisms at photosystem II may fall in stress conditions. Chlorophyll fluorescence correlates positively with carbohydrate concentration when carbohydrates maintain the integrity of chloroplast membranes by providing biosynthetic resources and stabilisation. Small differences in the Genty parameter, which was larger on the top of the dune, may indicate a small variability in the stress status of trees. The Genty parameter measured at low light was a reasonable approximation to the maximum photosystem II efficiency. The stress should be rather strong until larger differences in the maximum efficiency of photosystem II are observed. Fluorescence interpretation is complemented according to the chlorophyll concentrations (Lichtenthaler *et al.*, 2005). The smaller chlorophyll concentration at the higher elevations (Mandre & Korsjukov, 2003) points to differences in the density of photosynthetic units in needles. Therefore, one possibility is that the smaller chlorophyll concentrations were compensated by a higher efficiency of photosynthetic units.

The larger decrease of the photosynthetic efficiency after drying in laboratory in needles collected from the lower parts of the dune may indicate that on the top of the dune the trees tolerated possible severe drought conditions and were not weakened by the preceding winter. Numerous studies confirm that chlorophyll fluorescence analysis is a useful tool to measure drought tolerance of plants (Epron & Dreyer, 1993; Blödner *et al.*, 2005; O'Neill *et al.*, 2006; Percival *et al.*, 2006).

The methods of laboratory testing require further development. In this study the samples were consisted of an equal number of needles. Since the mass loss during drying was dependent on the initial sample mass, the results were confounded by the effect of the sample mass on drying. Apparently drying depended on the ratio of bag opening, which was constant, to the needle area and packing in the bags, which were variable. As the sample mass was correlated with the needle mass on trees, which in turn correlated with carbohydrates, the confounding effects and effects of interest were difficult to separate. Therefore, in future an even needle mass of samples should be used for the fluorescence analysis in the same way as in the present study.

Controlled conditions are used for the testing of plant material by fluorescence (Percival & Sheriffs, 2002). Use of very finely regulated individual chambers for each needle sample for simultaneous treatment of a large number of samples is currently unrealistic. The laboratory testing procedure should remain relatively simple. Probably it would be practical to dry needles without packing and in a climate chamber in a suitably humid air (Percival & Sheriffs, 2002; Muthuchelian *et al.*, 2005). Rewatering should be based on the individual water loss of the samples as excess water is damaging to the needles and its shortage may cause incomplete recovery.

Needle detachment may influence chlorophyll fluorescence and its response to the treatments of needles. Percival & Sheriffs (2002) found that ranking of woody species by a drought tolerance test was not affected by the detachment of leaves. Detachment gives different rankings by chlorophyll fluorescence in chilling experiments with grasses and vegetables (Potvin, 1985). For analysis of oak leaves humidified plastic bags have been used to gather the field samples and to measure the fluorescence within 3 h (Epron & Dreyer, 1993).

There are many factors that influence the needle carbohydrate concentration, photosystem II efficiency and shoot growth of pine on dunes. The spatial gradients of the factors change with time. A previous experiment showed that the effect of altered mineral nutrient composition is largest at the end of the growing season (Klůšeiko, 2006). During summer the trees could presumably suffer also from heating on the higher locations on the dune. In aspen heat tolerance depends on the leaf carbohydrate status (Hüve *et al.*, 2006).

Conclusions

The variability in carbohydrate concentrations of pine needles on a transect along diverse conditions on a dune was small. The hypothesis is proposed that stand development results in a balance between the carbohydrate production by trees and the consumption for growth and soil processes. On the top of the dune the low nutrient and water availability are in balance with the favourable light environment and slower growth of the trees, which allocate carbohydrates preferentially to roots. In undisturbed stands that have been growing in variable forest environments sufficiently long, carbohydrate concentrations in needles stabilize and differences in their concentration depend little on the site characteristics. The hypothesis could be tested by introducing forest thinning or nutrient treatments in stands on dunes and comparing these stands with untreated areas over longer period.

Carbohydrate concentrations and shoot morphology were significantly associated. Starch and sucrose concentrations were larger in shoots and needles with larger dry mass and the mass to length ratio.

Analysis of the photosystem II efficiency in detached needles collected during

drought in spring indicated that a small rain amount in May did not cause stress in the trees on the dune. Additional treatment by drying the needles in plastic bags increased the differences in the Genty parameter. This might indicate that the trees from the higher locations on a dune will tolerate drought better than the trees from the foot of the dune. However, to separate the confounding effects of sample handling procedures from the forest site effects the method of drying and recovery analysis with detached needles require further improvement with keeping the method simple and not reducing sample treatment capacity.

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Hariliku männi põuataluvus mitmekesistes kasvutingimustes luitel hinnatuna okaste süsivesikute sisalduse ning klorofüllil fluorestsentsi järgi

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Kokkuvõte

Süsivesikute sisaldus erinevused männiokastes olid väikesed, kuigi keskkonnatingimused ja puistu tihedus varieeruvad luitel oluliselt. Püstitati hüpotees, et puistu arengu jooksul süsivesikute moodustumine puudes ja kasutamine kasvuks ning mullaprotsessides tasakaalustuvad. Arvatavasti on luite tipus esinev vee ja mineraalainete raskendatud omastamine tasakaalustatud soodsate valgustingimuste poolt. Süsivesikute sisaldus okastes ja võrsete morfomeetrilised näitajad korreleerusid. Tärklise ja sahharoosi sisaldus oli suurem võrsetes ja okastes, millel oli suurem kuivmass. Fotosüsteem II kasuteguri analüüs klorofüllil fluorestsentsi (Genty parameetri) järgi näitas, et vähene sademete hulk maikuu ei põhjusta puudel stressi. Täiendav okaste kuivatamine laboritingimustes suurendas erinevusi Genty parameetri osas. Tulemuste põhjal oletati, et luite kõrgematel kohtadel paiknevad puud taluvad põuda paremini kui puud, mis asuvad luite jalamil. Siiski on klorofüllil fluorestsentsi analüüsi ja okaste laboratoorse töötlemise metoodikat vaja edasi arendada, kuna okaste kuivamine sõltus proovi massist. Täpsemate meetodite juures peab säilima praeguse metoodika kasutamise lihtsus ja analüüsikiirus.

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