BIOLOGIA PLANTARUM 55 (1): 90-98, 2011

Variability of photosynthetic capacity and water relations of *Pinus sylvestris* L. in the field

U. LÜTTGE[1]*, P. ESCHER[2], R. PALUCH[3], H. PFANZ[4], C. WITTMANN[4], H. RENNENBERG[2]
and K. RAKOWSKI[5]**

Institute of Botany, Darmstadt University of Technology, Schnittspahnstr. 3 - 5, D-64287 Darmstadt, Germany[1]
Institute of Forest Botany and Tree Physiology, University of Freiburg, D-79110 Freiburg, Germany[2]
Department of Natural Forest, Forest Research Institute, PL-17-230, Białowieża, Poland[3]
Institute of Applied Botany, University of Duisburg-Essen, Campus Essen, D-45117 Essen, Germany[4]
Forest Research Institute, PL-05-230 Raszyn, Poland[5]

Abstract

Measurements of dependence of photosynthetic electron transport on irradiance and analyses of stable isotope ratios ($\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$) were performed on 4 to 6-year-old pine trees (*Pinus sylvestris* L.) in the primeval forest reserve of Białowieża and on 21-year-old pine trees of a plantation of different provenances at the Sękocin Forest Station near Warsaw, Poland. Small differences in maximum photosynthetic electron transport rates, ETR_{max} were related to growth. Stable isotope analyses suggest that water relations play an important role for the performance of *P. sylvestris* at the sites studied. The intraspecific comparisons showed a very high variability of photosynthetic capacity between needles of given trees and between individual trees under similar conditions. Differences between specific provenances were also observed. This is relevant for ecological niche occupation in a wide geographical growth range, where *P. sylvestris* is actually occurring. The high physiological plasticity demonstrated reveals a conspicuous trait of this tree species.

Additional key words: chlorophyll fluorescence, quantum use efficiency, stable isotopes.

Introduction

In the literature, it is discussed controversially if photosynthetic capacity is a trait correlated to fitness (Ackerly *et al.* 2000) and productivity (Long *et al.* 2006). Still there are good reasons to expect that leaf photosynthesis is a good indicator of plant performance, also being a target for crop improvement (Long *et al.* 2006). In this context responses to stress are important even and especially so, if stress is only mild. Moreover, it is essential to look for possible intra-specific differences between genotypes albeit closely related. Santos *et al.* (2009) have performed such an analysis using five genotypes of common bean (*Phaseolus vulgaris*) under moderate water stress. They found differences in sensitivity of photosynthesis related parameters, such as stomatal conductance (g_s) and apparent electron transport rate (ETR). Differences in responses of chlorophyll fluorescence parameters were also observed among 5 cultivars of beech trees (Čaňová *et al.* 2008).

Asking such questions it appears to be appropriate to extend the range of intra-specific comparisons for which the species *Pinus sylvestris* L. (Scots pine) is chosen in the present investigation. Scots pine has a very large geographical distribution in Europe and Asia ranging from 8° to 114° east longitude and from 72° 20’ to 37° 00’ north latitude (Meusel *et al.* 1965, Chritchfield and Little 1966, Sokolov *et al.* 1977). This is connected with

---

Received 19 April 2009, *accepted* 17 December 2009.

Abbreviations: $\delta^{13}C$ - carbon isotope ratio; $\delta^{18}O$ - oxygen isotope ratio, $\delta^{15}N$ - nitrogen isotope ratio; ETR - apparent photosynthetic electron transport rate; ETR_{max} - ETR at saturation with PAR; F_0 - basic fluorescence of dark adapted needles; F_0’ - basic fluorescence of light adapted needles; F_m - maximum fluorescence of dark adapted needles; F_m’ - maximal fluorescence of light adapted needles; $\Delta F = F_m' - F_0'$; $\Delta F/F_m'$ - effective quantum yield of PS 2; $\Delta F/F_m'_{50}$ - $\Delta F/F_m'$ at half saturation; $\Delta F/F_m'_{sat}$ - $\Delta F/F_m'$ at saturation; $F_v = F_m - F_0$; F_v/F_m - potential quantum use efficiency; g_s - stomatal conductance; PAR - photosynthetically active radiation; PAR_{50} - half saturating PAR; PAR_{sat} - saturating PAR; PS - photosystem; RAPD - random amplified polymorphic DNA; WUE - water use efficiency.

Acknowledgements: We thank the administrations of the Primeval Forest Białowieża National Park and the Forest Research Institute Sękocin of the Instytut Badawczy Leśnictwa,Warsaw, for the permissions to work in the forests and for support on site. The editor-in-chief of Biologia Plantarum and an anonymous reviewer are thanked for very useful comments on the manuscript.

** Present address Pawlaczyka 1/7, PL-02-790 Warszawa, Poland
* Author for correspondence; fax: (+49) 6151164630, e-mail: luettge@bio.tu-darmstadt.de

the occurrence of many ecotypes of Scots pine forming stands both on dry and moist soils and also growing on both very poor and more fertile soils. Thus, among the ecophysiologically diverse populations there are considerable possibilities of intra-specific variability.

Studies on photosynthetic performance of plants in the field increasingly rely on measurements of photosynthesis using miniaturized equipment and on stable isotope analysis (Lüttge and Scarano 2004, 2007). Measurements of chlorophyll fluorescence parameters with a miniaturized pulse amplitude modulated (mini-PAM) fluorometer allow analyses of photosynthetic electron transport dependence on photosynthetically active radiation (PAR) for the assessment of maximum apparent electron transport rate of photosynthesis, ETR_{max}, and light saturation (PAR_{sat}). In contrast to these momentary measurements of chlorophyll fluorescence the PAR dependence curves provide an assessment of intrinsic photosynthetic capacity (Lüttge and Scarano 2007, Rascher *et al.* 2000). This is compared here with results of stable isotope analyses (^{18}O, ^{13}C and ^{15}N) of dried needles reflecting isotope discrimination over the life span of the needles. Interpretation of stable isotope discrimination of ^{18}O and ^{13}C allows conclusions on relations between stomatal opening and transpiration, and hence, on water relations (Farquhar *et al.* 1989 a,b, 1998, Barbour *et al.* 2002, Lüttge 2005) and ^{15}N can indicate nitrogen availability (Adams and Grierson 2001).

We wanted to know how large the intrinsic variability or plasticity of *P. sylvestris* might be. How large is the variability of performance between different trees at the same site as compared to effects of different external cues? Thus, we compared performance of individual trees at two different stands in the primeval forest reserve at Białowieża, Poland (Jędrzejewska and Jędrzejewski 1998) with different soil moisture regimes at defined sites and in an extended plantation of various provenances at the Sękocin forest station near Warsaw. The screening of many trees allowed characterization of variability between individual trees at a given site as well as the responses to environmental conditions at different stands of *P. sylvestris*.

Materials and methods

Selection of sites and trees: *Pinus sylvestris* L. is one of the most important forest trees in Europe where it covers 24 % of the forested area (Stanners and Bourdeau 1995, Oleksyn *et al.* 2002, Xiao *et al.* 2006). Measurements were performed in the Polish part of the nature reserve Primeval Forest Białowieża National Park and in the Forest Research Institute in Sękocin, Warsaw. The sites at Białowieża were *Vaccinio vitis-ideae-Pinetum* (plot #16) and a *Calamagrostio-Pinetum* (plot #5). The plot #16 is considered to be edaphically dry and plot #5 to be edaphically moist. We measured 10 and 12 young trees of *P. sylvestris* 4 to 6-year-old in plots #16 and #5, respectively.

At the Sękocin Station there is a plantation of 20 different provenances of *P. sylvestris* from all over Europe. The provenances are planted close to each other under identical edaphic and water table conditions. We selected 8 provenances for our study covering origins from different parts of Europe (see Szyp-Borowska and Staniolyte 2003, for details including exact geographical co-ordinates and altitudes). The numbers used here for identifying the provenances are the same as in their original publication. The trees were 21-year-old.

Photosynthetic capacity of leaves and conifer needles is age dependent (Warren 2006, Čaňová *et al.* 2008) and therefore all needles compared here were of the same age. They were measured and sampled within 10 d, *i.e.* from 28 June to 7 July 2005. All chlorophyll fluorescence measurements were made and samples for stable isotope analyses were taken of second year needles (needle year 2004). Measurements were made in the mid morning up to the afternoon (between 11:00 and 17:30). At Białowieża, fluorescence measurements were carried out directly on the young trees. At the Sękocin Station branches were cut from the exposed canopy on top of the trees and fluorescence measurements were performed on the cut branches on site immediately, *i.e.* up to 15 min after cutting.

Growth was measured by determining mean annual increment of main shoot axis length.

PAR dependence curves of photosynthetic electron transport: For each tree at Białowieża between 2 to 6 but mostly 5 samples were measured and for each tree at the Sękocin station always 3 samples were taken. The photosynthetic electron transport rates were measured with the *Mini-PAM fluorometer* (*H. Walz*, Effeltrich, Germany), as described earlier in detail (Rascher *et al.* 2000). A bunch of needles were clamped in the standard *2030-B* leaf clip holder of the instrument (Bilger *et al.* 1995). The PAR was incresed during 4 min in 8 steps at intervals of 30 s. In this way, instant PAR curves of effective quantum yield of photosystem 2 (PS 2), $\Delta F/F_m'$ = $(F_m' - F_0')/F_m'$ and apparent photosynthetic electron transport, ETR = 0.5 × 0.84 × $\Delta F/F_m'$ × PAR, were obtained, where F_0' is the basic fluorescence of the light adapted needles, F_m' is the maximum fluorescence of the light adapted needles under a saturating irradiance of 3000 $\mu mol\ m^{-2}\ s^{-1}$ for 800 ms, the factor 0.5 accounts for equal distribution of excitation energy to both PS 2 and PS 1, and the factor 0.84 considers that about 16 % of the incident irradiance are not absorbed (Schreiber and Bilger 1993). Measurements of PAR close to the needles were taken by the microquantum sensor of the leaf clip calibrated against a *LI-COR 190* quantum sensor (*LI-COR*, Lincoln, NE, USA).

In control runs, temperature of the needles was recorded during the measurements with intervals of 30 s up to 180 s between the 8 steps of increasing PAR. It was found that the mean temperature difference between the first and the last step was only 0.23 ± 0.93 °C. Thus, heating of the samples was negligible. Further, it was tested whether the short intervals of 30 s between the steps were adequate. This question was addressed using provenance #15 at the Sękocin Station. PAR dependence curves were performed by increasing the intervals between the steps from 30 to 180 s and comparing the relevant features of the curves obtained. Although the differences were rather small, at the extended intervals between steps the maximum ETR were significantly lower than at the shorter intervals. This implies that the needles already suffered some photoinhibition by the extended irradiance. Hence, the time interval of 30 s was a good choice and was used strictly in all recordings.

The PAR dependence curves were plotted from the measurements using *Sigma-Plot* with an exponential decay function for $\Delta F/F_m'$ and a hyperbolic saturation function for ETR. The following cardinal points describing the intrinsic capacity of the photosynthetic apparatus of the needles were obtained: ETR_{max}, $\Delta F/F_m'$ at saturation ($\Delta F/F_m'_{sat}$) and at half saturation ($\Delta F/Fm'_{50}$), saturating PAR (PAR_{sat}) and half saturating PAR (PAR_{50}).

Photoinhibition: Photoinhibition was assessed by measuring potential quantum use efficiency of PS 2, $F_v/F_m = (F_m - F_0)/F_m$, of needles adapted to darkness for 30 min, where F_0 is the basic fluorescence of the dark adapted needles and F_m is the maximum fluorescence of the dark adapted needles under a saturating PAR, and where values of F_v/F_m below 0.80 would indicate photoinhibition of the sample (Björkman and Demmig 1987). All curves were obtained between the late morning, noon and early afternoon when ambient irradiance was highest but the measurements of F_v/F_m showed that in all these measurements the needles never experienced photoinhibition.

Stable isotope analyses: Standard approaches according to Keitel *et al.* (2003) were used for determinations of C and N contents and analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of dried needles. Measurements were carried out with an elemental analyser (*NA 2500*, *CE Instruments*, Milan, Italy) coupled by a *Conflo II* interface (*Finnigan MAT*, Bremen, Germany) to an isotope ratio mass spectrometer (*Delta Plus*, *Finnigan MAT*). For oxygen ($\delta^{18}O$) isotope analyses a high temperature pyrolysis elemental analyser (*TC/EA*, *Finnigan MAT*) was coupled to the isotope ratio mass spectrometer. The isotope values are expressed in the standard delta notation (‰) relative to *VPDB* (*Vienna Pee Dee Belemnite*) for carbon, acetanilide and N_2 of air for nitrogen and *VSMO* (*Vienna Standard Mean Ocean Water*) for oxygen. The sample size of needles for stable isotope analyses was 3 for each tree at both sites.

Statistics: Statistical data analysis was performed with *SPSS 15.0* software (*SPSS*, Chicago, USA). Analysis of variance (*ANOVA*) using the general linear models procedure was employed for testing location effects on measured parameters. Prior to analysis, data were checked for normality and homogeneity of variance. Data were transformed, if necessary, to prevent violation of the *ANOVA* concept. In addition, statistical significance of differences between data sets was examined by Student's *t*-tests (*P* values are given in the text). In cases were averages of individual readings are presented the errors are given as standard deviations (SD), and where such averages were averaged the errors are given as standard errors (SE).

Results

Values of F_v/F_m are indicators for stress responses leading to photoinhibition. For all pine trees measured here at all sites values of F_v/F_m were always ≥ 0.80. The values measured were 0.83 - 0.84 at the dry site, 0.80 - 0.85 at the moist site of Białowieża and 0.83 - 0.85 at the Sękocin Station. Hence, there was no mid-day depression

Table 1. Averages of cardinal points from PAR dependence curves (see Materials and methods for abbreviations) of the trees at three different locations as indicated in the forest of Białowieża. Data are averages of the averages for all individual trees at each location with SE (number of trees in parenthesses). *ANOVA* results regarding location effects (dry/moist) on chlorophyll fluorescence data are shown.

Location	ETR_{max} [μmol m^{-2} s^{-1}]	$\Delta F/F_m'_{sat}$	$\Delta F/F_m'_{50}$	PAR_{sat} [μmol m^{-2} s^{-1}]	PAR_{50} [μmol m^{-2} s^{-1}]
Strict reserve	135 (2)	0.26 (2)	0.58 (2)	1237 (2)	272 (2)
Dry site	128 ± 7 (10)	0.25 ± 0.01 (10)	0.60 ± 0.01 (10)	1257 ± 74 (10)	260 ± 14 (10)
Moist site	149 ± 9 (12)	0.27 ± 0.01 (12)	0.58 ± 0.01 (12)	1350 ± 87 (12)	316 ± 21 (12)
df	1	1	1	1	1
F	3.389	2.625	10.827	0.624	4.332
Sig.	0.081	0.121	0.004	0.439	0.049

of photosynthesis. Needle temperatures during the measurements were recorded *via* the thermocouple of the leaf clip holder. The lowest and highest temperatures, respectively, occurring during the various runs of PAR dependence curves were 17/27 °C at the dry site and 21/29 °C at the wet site of Białowieża and 21/30°C at Sękocin. Thus, these temperature ranges closely overlapped. Temperatures also did not show any trends during the measurements, *i.e.* temperatures mainly scattered around the medium between the extremes indicated above. Since at mid-day the strongest stress due to irradiance and also temperature is expected, this means that the trees were never under photoinhibition at any time during the present study, *i.e.* none of the various mechanisms by which stress can possibly cause reduction of potential quantum yield were effective.

Saturating and half saturating PAR is rather high (Table 1 for Białowieża and Table 2 for Sękocin). Such demand of high irradiance for saturation of photosynthesis

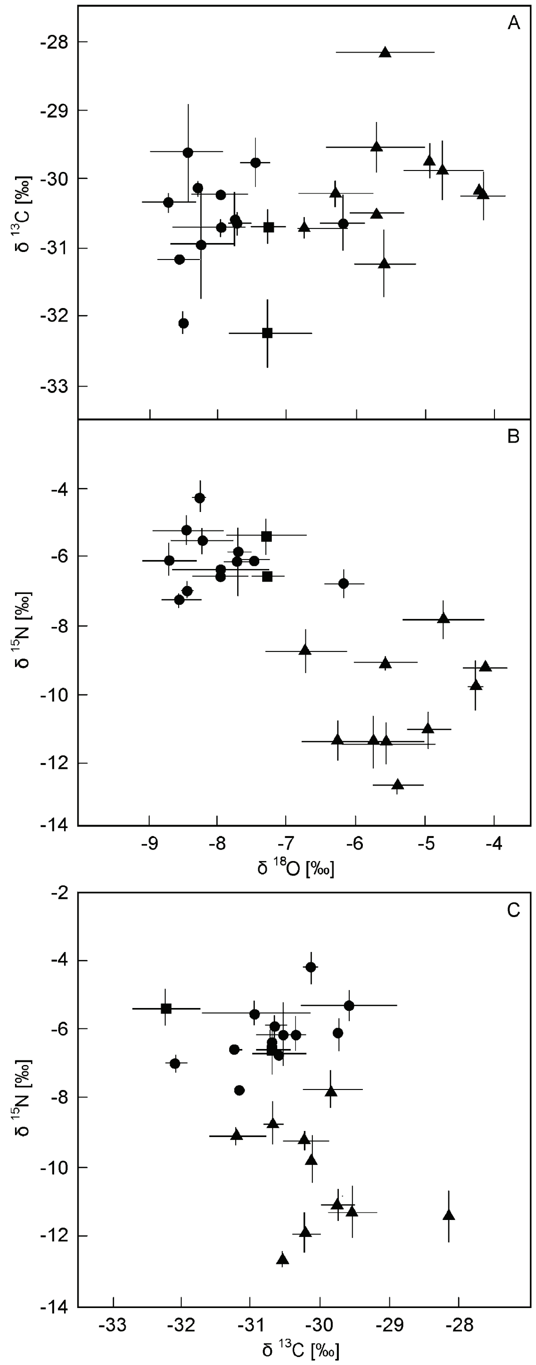

Fig. 1. Scatter diagrams of stable isotope ratios measured in three different samples ($n = 3$) from different trees (mean ± SD) at Białowieża (*squares* = strict reserve, *triangles* = dry site, *circles* = moist site).

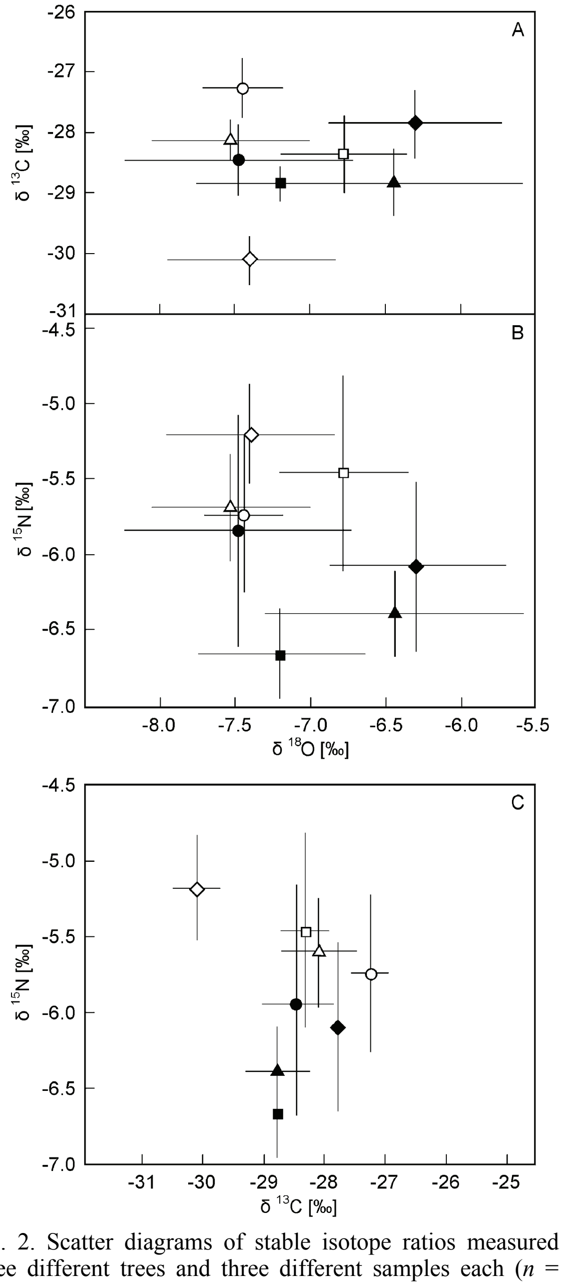

Fig. 2. Scatter diagrams of stable isotope ratios measured in three different trees and three different samples each ($n = 9$) from different provenances (mean ± SD) at Sękocin. Provenances are: *closed circles* - #2, *closed triangles* - #6, *closed squares* - #9, *closed rhombs* - #12, *open circles* - #13, *open triangles* - #15, *open squares* - # 17, *open rhombs* - #20.

Table 2. Averages of cardinal points from PAR dependence curves of the trees of 8 provenances of *P. sylvestris* at the Sękocin Station. Data are averages of the averages for all individual trees of each provenance with SE ($n = 3$, *i.e.* 3 individual trees for each provenance; numbers of measurements for each individual tree were 2 to 7).

Provenance	ETR_{max} [μmol m^{-2} s^{-1}]	$\Delta F/F_m{}'_{sat}$	$\Delta F/F_m{}'_{50}$	PAR_{sat} [μmol m^{-2} s^{-1}]	PAR_{50} [μmol m^{-2} s^{-1}]
#2	130 ± 13	0.22 ± 0.00	0.57 ± 0.01	1425 ± 125	276 ± 31
#6	136 ± 3	0.23 ± 0.01	0.56 ± 0.01	1444 ± 31	287 ± 8
#9	106 ± 11	0.20 ± 0.03	0.55 ± 0.03	1296 ± 86	233 ± 13
#12	87 ± 8	0.17 ± 0.00	0.55 ± 0.01	1211 ± 98	194 ± 19
#13	93 ± 6	0.19 ± 0.02	0.56 ± 0.01	1132 ± 45	193 ± 10
#15	131 ± 14	0.23 ± 0.02	0.56 ± 0.01	1388 ± 45	271 ± 25
#17	99 ± 13	0.18 ± 0.02	0.54 ± 0.04	1362 ± 23	231 ± 15
#20	108 ± 11	0.20 ± 0.01	0.56 ± 0.01	1350 ± 99	239 ± 23

Table 3. Range of maximum apparent photosynthetic electron transport rates (ETR_{max}) [μmol m^{-2} s^{-1}] in needles of individual trees at three different locations as indicated in the forest of Białowieża. Averages with SD ($n = 2$ to 6) are given for each tree. Mean for each location with SE ($n = 2$ to 12) is also given.

Strict reserve		Dry site		Moist site	
No.	ETR_{max}	No.	ETR_{max}	No.	ETR_{max}
1	147 ± 41	1	121 ± 18	1	154 ± 33
2	122 ± 19	2	131 ± 19	2	126 ± 34
mean	135	3	129 ± 29	3	187 ± 29
		4	147 ± 32	4	158 ± 47
		5	145 ± 12	5	124 ± 15
		6	106 ± 7	6	111 ± 13
		7	94 ± 17	7	108 ± 16
		8	109 ± 19	8	126 ± 25
		9	134 ± 27	9	181 ± 44
		10	182 ± 44	10	188 ± 51
		mean	130 ± 8	11	139 ± 23
				12	184 ± 0
				mean	149 ± 9

indicates a sun plant nature of the pine trees studied.

The variation of ETR_{max} among individual trees was larger than the average variation between locations in the forest (Table 3). The average ETR_{max} was a little but not significantly higher at the moist site than at the dry site and the location in the strict reserve ($P = 0.1$). Averages of all cardinal points extracted from all individual PAR dependence curves obtained at each location (Table 1) show a slightly better performance with respect to ETR_{max} and $\Delta F/F_m{}'_{sat}$ at the moister site than at the drier site. This is correlated with growth as given by annual increments of the height of the main stem axis, which for all years was significantly ($P < 0.001$) higher in the moist than in the dry site (Table 4). The increments in stem length were proportionally much larger (1.91 to 2.84 fold in the various years) than the increase in ETR_{max} (1.15 fold) in the moist site as compared to the dry site. However, one would not expect a similar factor and small differences in ETR_{max} may be important in the field where small increases in photosynthesis may add up to an important contribution over time.

Stable isotope data are related to each other. It is seen for the scattered data of individual trees and the averages that $\delta^{18}O$ was more negative in the moist site and the strict reserve than in the dry site (Fig. 1 and Table 5, $P < 0.001$). $\delta^{13}C$ values were not different at the three different sites. $\delta^{15}N$ values were less negative in the moist site and strict reserve than in the dry site ($P < 0.001$).

Table 4. Growth rates of the trees in the forest of Białowieża as given by means of main shoot increments in different years. Values are in cm with SE of 30 trees. Differences between the locations (dry/moist) as examined by Student's *t*-tests for all years are significant at the level of $P < 0.001$.

Year	2005	2004	2003	2002	2001
Dry site	18.9±0.9	14.2±0.9	15.1±1.1	12.9±1.3	10.1±0.8
Moist site	37.7±1.7	35.3±1.8	28.8±1.3	27.8±1.5	28.7±1.5

Table 5. Averages of stable isotope data [‰] of the trees at three different locations as indicated in the forest of Białowieża. *ANOVA* results regarding location effects (dry/moist) on isotope data are shown

Location	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$
Strict reserve	-7.28 ± 0.41	-31.5 ± 0.9	-5.99 ± 0.73
Dry site	-5.35 ± 0.67	-30.0 ± 0.8	-10.2 ± 1.53
Moist site	-7.96 ± 0.69	-30.7 ± 0.7	-6.13 ± 0.90
df	1	1	1
F	65.5	3.89	60.7
Sig.	0.000	0.063	0.000

Contents of N and C/N ratios did not differ between the pine trees at the different plots and averages were 2.3 % N and 22.3 C/N.

At the plantation site of Sękocin variations of ETR_{max} between individual trees of each provenance were larger than variations between different provenances (Table 6). Nevertheless, it is evident that some provenances had a lower intrinsic photosysnthetic capacity than others, *e.g.* provenances #9, #12, #13, #17 and #20 had lower ETR_{max} than provenances #2, #6 and #15 ($P < 0.05$ to < 0.001). Averages of all cardinal points (Table 2) again indicate a better performance of provenances #2, #6 and #15 with respect to ETR_{max} and $\Delta F/F_{m}{}'_{sat}$ as compared to the other provenances.

For $\delta^{18}O$ the values were more negative in #13 and #15 than in #12 ($P = 0.02$). For $\delta^{15}N$ the values in #13, #15, #17 and #20 were less negative than in #9 ($P = 0.001$). For $\delta^{13}C$ the values in #20 were more negative than in all other provenances ($P < 0.02$ to < 0.001), the values in #13 were less negative than in #2, #6, #9, #15, #17 and #20 ($P < 0.05$ to < 0.001) and the values in #12 were less negative than in #9 ($P < 0.001$) (Fig. 2, Table 7). There was no correlation of bulk N content and C/N ratios with photosynthetic performance. Contents of N and C/N ratios did not differ significantly between provenances and averages were 2.4 % N and 20.8 C/N, which did not differ significantly from those observed in Białowieża.

Table 6. Range of maximum apparent photosynthetic electron transport rates (ETR_{max}) in needles of three individual trees each of 8 provenances of *P. sylvestris* cultivated at the Sękocin Station. Averages with SD (*n* = 2 to 7) are given for each tree. Means for each provenance with SE (*n* = 3) are also given.

	ETR_{max}		ETR_{max}		ETR_{max}		ETR_{max}
#2	147 ± 34	#9	84 ± 42	#13	100 ± 16	#17	78 ± 15
	138 ± 77		119 ± 50		99 ± 22		95 ± 28
	104 ± 26		115 ± 14		81 ± 18		124 ± 0
Mean	130 ± 13		106 ± 11		93 ± 6		99 ± 13
#6	138 ± 82	#12	102 ± 13	#15	150 ± 61	#20	92 ± 17
	137 ± 55		81 ± 25		141 ± 23		128 ± 21
	132 ± 44		78 ± 15		103 ± 18		103 ± 43
Mean	136 ± 3		87 ± 8		131 ± 14		108 ± 11

Table 7. Averages of stable isotope data [‰] for the trees of 8 provenances at the Sękocin Station. All values are with SD; *n* = 9 per provenance, *i.e.* 3 trees per provenance and 3 samples per tree.

Provenance	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$
#2	-7.48 ± 0.76	-28.42 ± 0.59	-5.84 ± 0.77
#6	-6.45 ± 0.87	-28.83 ± 0.54	-6.38 ± 0.29
#9	-7.20 ± 0.56	-28.78 ± 0.11	-6.66 ± 0.30
#12	-6.30 ± 0.59	-27.79 ± 0.11	-6.08 ± 0.56
#13	-7.45 ± 0.27	-27.25 ± 0.33	-5.74 ± 0.52
#15	-7.53 ± 0.53	-28.12 ± 0.63	-5.59 ± 0.36
#17	-6.78 ± 0.43	-28.33 ± 0.41	-5.45 ± 0.64
#20	-7.40 ± 0.56	-30.08 ± 0.41	-5.19 ± 0.34

Discussion

Photosynthetic parameters, such as ETR_{max}, have been used in evaluating intrinsic photosynthetic capacity and performance of plants (Rascher *et al.* 2000, Lüttge and Scarano 2004). The present study shows the usefulness of this approach in combination with stable isotope data for assessing the plasticity of ecophysiological performance of pine trees with respect to water relations. Better performance regarding ETR_{max} is related to a better water availability. This is seen in comparing the dry and moist site at Białowieża and considering the measurements of the provenances at the Sękocin Station, where 5 provenances (#9, #12, #13, #17, #20) showed ETR_{max} values even lower than in the trees at the dry site in Białowieża. This can be explained by the fact that annual precipitation is higher in Białowieża (626 mm) than at Sękocin (529 mm).

Foliar $\delta^{13}C$ is considered an indicator of water use efficiency (WUE) during the life span of the leaves or needles sampled, or even longer. In the latter case $\delta^{13}C$ of leaves depends on $\delta^{13}C$ of carbon storage pools remobilised during bud break, carbon assimilated during leaf expansion and assimilates produced subsequently in the growing season (Farquhar *et al.* 1989 a,b, 1998, Duquesnay *et al.* 1998, Warren *et al.* 2001). Therefore, carbon isotope ratios have been used for assessing long term water relations of plants performing C_3 photosynthesis, where more negative values of carbon isotope ratios are proportional to increased stomatal conductance (g_s), decreased WUE, and an increase in the ratio of internal to external CO_2 partial pressure, c_i/c_a. However, $\delta^{13}C$ is also modulated by mesophyll conductance, which – although potentially also varying intraspecifically – mostly affects comparisons between different species (Flexas *et al.* 2008). Furthermore it is affected by metabolic isotope effects related to photosynthesis as well as respiration (Kodama *et al.* 2008). An increase in c_i/c_a, as deduced from more negative $\delta^{13}C$ values (Farquhar *et al.* 1989a,b) may be either due to an increase in g_s and lower WUE at constant maximum CO_2 assimilation or *vice versa* due to a decrease of CO_2 assimilation at constant g_s (Scheidegger *et al.* 2000). Thus, it is important to note that in the present investigation $\delta^{13}C$ values were not different in the different trees in Białowieża.

However, the assessment of water use efficiency is facilitated if stable oxygen isotope ratios can be considered in addition. A reduced discrimination against ^{18}O depends on the ratio of the partial water vapour pressures in the atmosphere and in the gaseous space within the needles and is a function of the back-diffusion of water from sites of evaporation being opposed by convection of source water to these sites *via* transpiration (Farquhar *et al.* 1998, Barbour *et al.* 2002). This so called Péclet effect is given by the ratio of non-enriching convection of water to the leaf evaporative sites *via* the transpiration stream to the effect of ^{18}O-enriching diffusion of water away from the sites of evaporation (Barnard *et al.* 2007). Stable oxygen isotope ratios reflect leaf water enrichment, and while there is no ^{18}O enrichment in the xylem stream water, δ^{18}O in the organic matter of bulk leaf material is a useful tool to characterise stomatal conductance independent of effects of photosynthesis over the life span of the leaves or needles (Adams and Grierson 2001). Like stable carbon isotope ratios, stable oxygen isotope ratios result from several parameters (Brandes *et al.* 2007). Nevertheless, the observation that at the two close-by sites at Białowieża δ^{18}O is more negative in the moist site suggests that transpiration is higher than in the dry site (Table 5). This supports the correlation that water availability determines performance as ETR$_{max}$ is higher at the moist site than at the dry site (Table 1).

Due to the variation between individual provenances an overall comparison becomes somewhat less straightforward if we take the data of the Sękocin Station into consideration. The δ^{13}C values at the Sękocin Station with the lower annual precipitation except for provenances #6 and #20 are less negative than the values at all Białowieża sites with the higher annual precipitation which would be in accordance with a reduced transpiration and higher stomatal control at the drier Sękocin Station. However, the δ^{18}O values at the Sękocin Station are more negative and closer to those of the moist site than the dry site suggesting that transpiration on average in the Sękocin Station is as high as in the moist site at Białowieża. If we compare the Sękocin Station with Białowieża we must bear in mind, however, that in addition to the different performances of the various provenances at the Sękocin Station we also have the problem that we compare differences in pedosphere relations for the two plots at Białowieża and differences of annual precipitation if we compare the Sękocin Station with Białowieża. Therefore, in view of the multifactorial effects on stable isotope ratios we cannot put too much weight on this comparison. Moreover, with the large geographical range of Scots pine (see Introduction) many ecotypes developed which show different degrees of resistance to water shortage, so that there are also putative genotype differences between provenances some of which are susceptible while others are resistant to drought (Birkjukova *et al.* 1981). Evidently the differences between provenances cannot be explained by climatic and edaphic site characteristics including water tables (Gessler *et al.* 2005) as possible cause.

However, genotype effects must be subtle. The photosynthetic capacities as given by ETR$_{max}$ of the different provenances do not appear to be related to their gross relative genetic distance. Provenances with higher (#2, #6, #15) and with lower ETR$_{max}$ (#9, #17, #13), respectively, are randomly distributed along a genetic tree obtained by random amplified polymorphic DNAs (RAPD; Szyp-Borowska and Staniulyte 2003), although we must admit that RAPD is not considered an up to date technique anymore. It is also hard to estimate if the performance given by the measurements of ETR$_{max}$ is related to the geographic origin of the provenances. Two of the better performing provenances (#2, #15) are from northern Europe and the third one is from eastern Poland (#6), while the less well performing ones are from western Poland, western Hungary and Western Europe and one (#20) is from Turkey at 1400 m a.s.l.

In literature in general (Evans 1988, Field 1988) and, in particular from investigations with conifers (Duursma and Marshall 2006), it is known that maximum rates of CO_2 uptake by leaves or needles are correlated with nitrogen content. Some relations to N supply *via* transpiration are also seen in the present data when we compare the moist and dry sites at Białowieża. Although bulk N content and C/N-ratios were not different at the two sites δ^{15}N values were much more negative at the dry site where δ^{18}O values were less negative and transpiration must have been lower than at the moist site where these relations were the opposite (Table 5). The degree of transpiration appears to affect allocation of nitrogen compounds to the needles. This is also indicated by the trend of less negative towards more negative δ^{15}N-values *versus* more negative towards less negative δ^{18}O-values (or lower transpiration) seen in Fig. 1. Thus, it may be assumed that N supply is involved in the interdependency of increased transpiration and increased ETR$_{max}$.

Conclusions

A broad geographic scale comparison of Scots pine all across Eurasia suggests that *P. sylvestris* has higher transpiration rates at drier and warmer sites at the expense of lower water-use efficiency (Poyatos *et al.* 2007). The data presented here support the contention that the ecophysiological comportment of *P. sylvestris* is closely related to moisture. Photosynthetic capacity is affected by water relations and therefore is an important trait in relation to the performance of *P. sylvestris* in forest ecosystems and plantations.

The most conspicuous result of the present study is the large variability of the photosynthetic capacity and performance of individual trees of *P. sylvestris* at a given site or of a given provenance. The considerable amplitude of photosynthetic performance observed among the Scots pine trees evidently shows high ecophysiological variability. This is relevant for ecological niche occupation in a wide geographical growth range, where *P. sylvestris* is actually occurring.

References

Ackerly, D.D., Dudley, S.A., Sultan, S.E., Schmitt, J., Coleman, S., Linder, C.R., Sandquist, D.R., Geber, M.A., Evans, A.S., Dawson, T.E., Lechowicz, M.J.: The evolution of plant ecophysiological traits: recent advances and future directions. - BioScience **50**: 979-995, 2000.

Adams, M.A., Grierson, P.F.: Stable isotopes at natural abundance in terrestrial plant ecology and ecophysiology: an update. - Plant Biol. **3**: 299-310, 2001.

Barbour, M.M., Walcroft, A.S., Farquhar, G.D.: Seasonal variation in $\delta^{13}C$ and $\Delta^{18}O$ of cellulose from growth rings of *Pinus radiata*. - Plant Cell Environ. **25**: 1483-1499, 2002.

Barnard, R.L., Salmon, Y., Kodama, N., Sörgel, K., Holst, J., Rennenberg, H., Gessler, A., Budemann, N.: Evaporative enrichment and time lags between $\delta^{18}O$ of leaf water and organic pools in a pine stand. - Plant Cell Environ. **30**: 539-550, 2007.

Bilger, W., Schreiber, U., Bock, M.: Determination of quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. - Oecologia **102**: 425-432, 1995.

Birjukova, Z.P., Harlamova, N.: [Geographical variavility of drought and cold resistance of common pine.]. - Ekologiya **4**: 42-47, 1981. [In Russ.]

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. - Planta **170**: 489-504, 1987.

Brandes, E., Wenninger, J., Koeniger, P., Schindler, D., Rennenberg, H., Leibundgut, C., Mayer, H., Gessler, A.: Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. - Plant Cell Environ. **30**: 113-127, 2007.

Čaňová, I., Ďurkovič, J., Hladká, D. Stomatal and chlorophyll fluorescence characteristics in European beech cultivars during leaf development. - Biol. Plant. **52**: 577-581, 2008.

Critchfield, W.B., Little, E.L.: Geographic Distribution of Pines of the World. - US Dept. Agricult. Forest Service, Washington 1966.

Duquesnay, A., Bréda, N., Stievenard, M., Dupouey, J.L. Changes of tree-ring $\delta^{13}C$ and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. - Plant Cell Environ. **21**: 565-572, 1998.

Duursma, R.A., Marshall, J.D.: Vertical canopy gradients in $\delta^{13}C$ correspond with leaf nitrogen content in a mixed-species conifer forest. - Trees **20**: 496-506, 2006.

Evans, J.R.: Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. - Aust. J. Plant Physiol. **15**: 93-106, 1988.

Farquhar, G.D., Barbour, M.M., Henry, B.K.: Interpretation of oxygen isotope composition of leaf material. - In: Griffiths, H. (ed.): Stable Isotopes – Integration of Biological, Ecological and Geochemical Processes. P. 27. BIOS Sientific, Oxford 1998.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T.: Carbon isotope discrimination and photosynthesis. - Annu. Rev. Plant Physiol. **40**: 503-537, 1989a.

Farquhar, G.D., Hubick, K.T., Condon, A.G., Richards, R.A.: Carbon isotope fractionation and plant water-use efficiency. - In: Rundel, P.W., Ehleringer, J.R., Nagy, K.A. (ed.): Stable Isotopes in Ecological Research. Pp. 21-40. Springer-Verlag, Berlin - Heidelberg - New York 1989b.

Field, C.B.: On the role of photosynthetic responses in constraining the habitat distribution of rainforest plants. - Aust. J. Plant Physiol. **15**: 343-358, 1988.

Flexas, J., Ribas-Carbó, M., Diaz-Espejo, A., Galmés, J., Medrano, H.: Mesophyll conductance to CO_2: current knowledge and future prospects. - Plant Cell Environ. **31**: 602-621, 2008.

Gessler, A., Duarte, H.M., Franco, A.C., Lüttge, U., De Mattos, E.A., Nahm, M., Scarano, F.R., Zaluar, H.L.T., Rennenberg, H.: Ecophysiology of selected tree species in different plant communities at the periphery of the Atlantic Forest of SE-Brazil. II. Spatial and ontogenetic dynamics in *Andira legalis*, a deciduous legume tree. - Trees **19**: 510-522, 2005.

Jędrzejewska, B., Jędrzejewski, W.: Predation in Vertebrate Communities. The Białowieża Primeval Forest as a Case Study. - Springer, Berlin - Heidelberg - New York 1998.

Keitel, C., Rennenberg, H., Adams, M.A., Gessler, A.: Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L). - Plant Cell Environ. **26**: 1157-1168, 2003.

Kodama, N., Barnard, R. L., Salmon, Y., Weston, C., Ferrio, J. P., Holst, J., Werner, R.A., Saurer, M., Rennenberg, H., Buchmann, N., Gessler, A.: Temporal dynamics of the carbon isotope composition in a *Pinus sylvestris* stand: from newly assimilated organic carbon to respired carbon dioxide. - Oecologia **56**: 737-750, 2008.

Long, S.P., Zhu, X.-G., Naidu, S.L., Ort, D.R.: Can improvement in photosynthesis increase crop yields? - Plant Cell Environ. **29**: 315-330, 2006.

Lüttge, U.: Physiologische Ökologie der Photosynthese – Autökologische und synökologische Aspekte anhand von $\delta^{13}C$- und $\delta^{18}O$-Daten. - In: Auf Spurensuche in der Natur. P. 69. Dr. Friedrich Pfeil, München 2005.

Lüttge, U., Scarano, F.R.: Ecophysiology. - Rev. Brasil Bot. **27**: 1-10, 2004.

Lüttge, U., Scarano, F.R.: Synecological comparisons sustained by ecophysiological fingerprinting of intrinsic photosynthetic capacity in plants as assessed by measurements of light response curves. - Rev. Brasil Bot. **30**: 355-364, 2007.

Meusel, H., Jäger, W., Weinert, E.: Vergleichende Chorologie der zentraleuropäischen Flora 1. G. Fischer, Jena 1965.

Oleksyn, J., Reich, P.B., Zytkowiak, R., Karolewski, P., Tjoelker, M.G.: Needle nutrients in geographically diverse *Pinus sylvestris* L. populations. - Ann. Forest. Sci. **59**: 1-18, 2002.

Poyatos, R., Martínez-Vilalta, J., Čermák, J., Ceulemans, R., Granier, A., Irvine, J., Köstner, B., Lagergren, F., Meiresonne, L., Nadezhdina, N., Zimmermann, R., Llorens, P., Mencuccini, M.: Plasticity in hydraulic architecture of Scots pine across Eurasia. - Oecologia **153**: 245-259, 2007.

Rascher, U., Liebig, M., Lüttge, U.: Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. - Plant Cell Environ. **23**: 1397-1405, 2000.

Santos, M.G., Ribeiro, R.V., Machado, E.C., Pimentel, C.: Photosynthetic parameters and leaf water potential of five common bean genotypes under mild water deficit. - Biol. Plant. **53**: 229-236, 2009.

Scheidegger, Y., Saurer, M., Bahn, M., Siegwolf, R.: Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. - Oecologia **125**: 350-357, 2000.

Schreiber, U., Bilger, W.: Progress in chlorophyll fluorescence research: major developments during the last years in retrospect. - Prog. Bot. **54**: 151-173, 1993.

Sokolov, S.J., Svjazeva, O.A., Kubly, V.A.: Arealy Derevev i Kustarnikov SSSR. 1. [Areas of Trees and Shrubs in USSR. 1.] - Nauka, Leningrad 1977. [In Russ.]

Stanners, D., Bourdeau, P.: Europe's Environment: the Dobris Assessment Report. - Europe's Environment Agency, Copenhagen 1995.

Szyp-Borowska, I., Staniulyte, R.: [Use of RAPD markers in evaluation of genetic diversity of european populations of *Pinus sylvestris.*] - Biotechnologica **2**: 280-289, 2003. [In Polish]

Warren, C.R.: Why does photosynthesis decrease with needle age in *Pinus pinaster*? - Trees **20**: 157-164, 2006.

Warren, C. R., McGrath, J.F., Adams, M.A.: Water availability and carbon isotope discrimination in conifers. - Oecologia **127**: 476-486, 2001.

Xiao, C.-W.X., Janssens, I.A., Yuste, J.C., Ceulemans, R.: Variation of specific leaf area and upscaling to leaf area index in mature Scots pine. - Trees **20**: 304-310, 2006.