Phyton (Austria) Special issue: "D. Grill"	**Vol. 45**	Fasc. 3	(195)-(212)	1.9.2005

Light-Modulation of Corticular CO_2-Refixation in Young Birch Stems (*Betula pendula* Roth.)

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K e y w o r d s : *Betula*, stem respiration, carbon gain, CO_2-refixation, corticular photosynthesis, CO_2 efflux.

S u m m a r y

WITTMANN C., PFANZ H. & PIETRINI F. 2005. Light-modulation of corticular CO_2-refixation in young birch stems (*Betula pendula* Roth.). - Phyton (Horn, Austria) 45 (3): (195)-(212).

Photosynthetic bark reduces the flux of CO_2 from woody tissues to the atmosphere by recycling endogenous CO_2 derived from mitochondrial respiration. In young birch trees (*Betula pendula* Roth.) this process is modulated by the light intensity regime at the growing site. Although positive net photosynthesis was not yet found in intact birch twigs, CO_2 efflux was distinctly reduced upon illumination by 65% in high-light grown birches (HL: 100% sunlight) and by up to 82% in low-light grown trees (LL: 20% of full sunlight).

Bark chlorenchymes were found to be optimized for light harvesting and photosynthetic performance under LL-conditions, due to the shading effect of the outer peridermal (or rhytidomal) layers. Compared on a unit area basis the inner bark tissue contained up to 47%-49% of the chlorophyll of the concomitant leaves under both HL and LL conditions, respectively.

Peridermal light transmittance was changed by the light intensity regime during growth, due to changes within the microstructure of the outer bark. The inner bark morphology, quantified as specific bark area (SBA), was found to be strongly correlated with twig respiration (r^2 = 0.83-0.96) and the CO_2-refixation rate (r^2 = 0.81-0.95) under LL and HL conditions and thus can be used to predict the efflux of CO_2 from the stem organs.

Besides leaf dark respiration (R_d) also twig R_d was clearly related with the light environment. Under shading the investigated twigs showed a lower R_d but a higher relative CO_2-refixation (expressed as a percentage of dark respiration); up to 100% of the respired carbon was refixed in LL twigs. Furthermore, twig R_d was the physiological parameter that correlated most strongly with CO_2-refixation rate.

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Introduction

Light is one of the major environmental factors regulating plant productivity. Therefore tuning of foliar anatomical and biochemical features to the light gradient in the canopy plays a major part in optimizing canopy photosynthesis (BJÖRKMAN 1981, EVANS 1989, PEARCY & SIMS 1994).

Aside from the leaves, a chlorophyll-containing bark tissue can be found even in the lignified stems and branches of nearly all trees, shrubs and bushes. Several authors have demonstrated that the bark chlorenchyme in woody trees is able to photosynthetically reduce the flux of respiratory CO_2 to the atmosphere and in parallel to evolve thylakoid-borne O_2 (FOOTE & SCHAEDLE 1976, PFANZ & ASCHAN 2000, PFANZ & al. 2002, PILARSKI 2002), a process that has been termed „CO_2-refixation" or alternatively „corticular photosynthesis" (SPRUGEL & BENECKE 1991, NILSEN 1995). The prerequisites necessary for a working reductive CO_2 assimilation metabolism (e.g. an effective chloroplast structure, enzymatic equipment, nutrients, water, light and carbon dioxide) were shown to be present in sufficient amounts and quantities within the chlorenchymal bark tissues of trees (PFANZ & al. 2002).

Plant productivity depends on the balance between photosynthetic carbon assimilation and the expenditure of fixed carbon by respiration (EDWARDS & al. 1981, WARING & RUNNING 1998). Equivalent gains in whole plant carbon assimilation can consequently be made by increasing the rate of carbon uptake from the atmosphere (e.g. by the stimulation of leaf photosynthesis) or by decreasing respiratory carbon losses (e.g. through corticular photosynthesis; CERNUSAK & MARSHALL 2000). Stem respiration is estimated to range from 13% (RYAN & WARING 1992) to as much as 42% (WARING & SCHLESINGER 1985) of the total aboveground carbon budget in mature forests. Respiration is therefore regarded as a main factor in the regulation of forest productivity and carbon storage (RYAN & al. 1997, DAMESIN & al. 2002). As plant respiration is a significant component of the global C cycle, changes in plant respiration would alter the ecosystem carbon balance (RYAN 1991).

In different deciduous trees (e.g. *Fagus sylvatica*, *Populus tremula*) refixation of CO_2 within twigs and branches was shown to compensate for 60-90% of the potential respiratory carbon loss (FOOTE & SCHAEDLE 1976, WITTMANN & al. 2001, ASCHAN & al. 2001). The light-driven refixation of respiratory carbon dioxide may therefore be an important strategy of additional carbon-acquisition.

Studies of metabolic and photosynthetic activity in trees have traditionally focused on leaves as primary indicators of whole-tree carbon assimilation, whereas little work has been done on the gas exchange of woody tissues. Despite the importance of woody tissue respiration, that may even equal or exceed foliar respiration on a whole-tree or stand basis (EDWARDS & HANSON 1996). It is surprising that there are only a few studies on respiration that provide data about the light-driven refixation of respiratory carbon dioxide by corticular photosynthesis.

We wanted: (1) to illuminate the contribution of corticular photosynthesis to the carbon balance of birch trees; (2) to determine the influence of the light envi-

ronment on the photosynthetic activity of the inner bark cells; (3) to study anatomical and physiological factors that may effect or even regulate CO_2-refixation. Therefore we exposed young birch trees (*Betula pendula* Roth., 6-year-old) to distinct light environments (LL: 20% and HL: 100% of full sunlight) and studied the effectiveness and the probable adaptive light dependency of stem-internal carbon refixation as well as related morphological and physiological traits.

Material and Methods

Plant material

6-year-old birch trees (*Betula pendula* Roth.) were grown outside in 20 L plastic containers under sufficient nutrition (Einheitserde Typ T, Balster, Germany) and water supply, realised by periodic fertilisation with Osmocote (Bayer, Germany) and daily irrigation. In early spring 1999, 20 trees were shaded with a tentlike construction, covered with a light-reducing net tissue (light permeability about 20% of incident sunlight); another 20 trees (control, 100%) were exposed to natural sunlight. The net structure of the shading tissue as well as the open front and back of the tent enabled permanent air circulation, avoiding overheating during hot summer days.

Optical properties of the outer bark layers

According to PFANZ 1999 the outer bark layers were removed carefully with a cork borer. Absorptance, reflectance and transmittance spectra between 400 and 1100 nm were obtained by a LI-COR hand-held spectroradiometer, model Li-1800, connected to an external LI-1800-12 integrating sphere by means of a quartz fiberoptic probe (LI-COR, Inc., Lincoln, NE, USA). A 10 W glass halogen lamp, connected to the integrating sphere, served as the radiation source. The measurement configuration was changed sequentially according to the manufactor's instructions for recording reflectance and transmittance spectra from a $1cm^2$ surface (sensor area) and from a pressed barium sulphate reference standard. Absorptance (A) over the waveband 400-1100 nm was calculated at each wavelength (λ) as $A_{(\lambda)} = 100-(R_{(\lambda)} + T_{(\lambda)})$. R was calculated as a percentage of I_l/I_s where I_l was the measured sphere output when the leaf was illuminated and I_s the output measure when the pressed barium sulphate reference standard was illuminated; T was obtained by the percentage of I/I_o where I was the measured sphere output when radiation was transmitted through the sample and I_o was the measured sphere output when radiation did not pass through the sample and the reference material was illuminated. Total periderm absorptance was compensated for the spectral composition of the light source using the equation (1):

$$A_{400\text{-}1100nm} = \Sigma_{(400\text{-}1100)}\ A_{(\lambda)} * \in_{(\lambda)} \qquad \text{eqn. (1)}$$

where $A_{(\lambda)}$ is the absorptance at a given wavelength (λ) and $\in_{(\lambda)}$ is the relative emittance of the sorce at the same wavelength.

Photosynthetic pigment determination

Photosynthetic pigments were determined in tissue disks removed by a cork borer (5 mm in diameter) and placed in 80% (v/v) DMSO (dimethyl sulfoxide). Pigment extraction required approximately 2 h at 65°C in the dark (e.g. BARNES & al. 1992). To avoid acidification and a concomitant phaeophytinisation of the chlorophylls, 20 mg $Mg_2(OH)_2CO_3$ was added. Finally, extract absorbances were measured with a spectrophotometer (UV 160, Shimadzu, Japan) and pigment contents calculated according to standard equations (WELLBURN 1994).

Specific leaf and bark area and mass measurements

Projected leaf area of foliage samples was measured using an area meter (ADC, Area Meter AM 100). Foliage samples were then dried at 70°C for 48 h and weighed. For the determination of SBA (specific bark area) a one cm^2 piece of bark from young twigs was removed using a cork borer, dried for 48 h at 70°C and weighed.

Gas exchange measurement

Gas exchange measurements of intact twigs and leaves were made with a portable porometer system (model Li-6400, Li-Cor Inc., Lincoln, USA). To avoid possible wound respiration only intact twig internodes or single mature leaves were selected for the gas-exchange measurements. Dark respiration was measured after a 30-min shading period of the respective plant parts within the cuvette. Subsequent light response curves were conducted under constant climatic conditions (20°C, 50-55% relative humidity) and a controlled CO_2 supply (350 ppm). High-irradiance CO_2 - exchange rates of the twigs were first measured after a 30-min period of light exposure to 1500 µmol photons m^{-2} s^{-1}. At least ten independent light response curves were assessed for each type of leaf or twig internode.

Light saturation of CO_2-assimilation was defined at 90% of maximum photosynthetic rate (e.g. VON WILLERT & al. 1995). Stem internal CO_2-refixation rates were calculated as the difference between CO_2 efflux in the dark and under maximum illumination with 1500 µmol photons m^{-2} s^{-1}. Statistical analysis of the light response curves showed that the relationship can be described most appropriate by the exponential equation:

$$P_{re} = R_d + P_{re\,(max)} [1-\exp(-\alpha(I)], \qquad \text{eqn. (2)}$$

were P_{re} is photosynthetic CO_2-refixation rate, R_d is dark respiration rate, $P_{re\,(max)}$ is the light-saturated rate of CO_2-refixation, α is the initial slope or quantum efficiency of the light response curve, and I is the amount of PAR reaching the outer bark surface. Relative refixation as percentage of dark respiration rate was estimated from CO_2 efflux in the dark (R_d) and under illumination with 1000 µmol PAR m^{-2} s^{-1} (R_{il}) as follows:

$$\text{rel. Refixation [\% of dark respiration]} = [(R_d-R_{il})/R_d] \times 100 \qquad \text{eqn. (3)}$$

Statistical analysis, calculations and curve fittings were done using Sigma Plot 5.0 (SPSS Science Software).

Results and Discussion

Peridermal PFD transmission

The penetration of light into stems and leaves is clearly different. While in leaves the light has to pass only the living epidermal cells (aside from thin layers of wax and cuticles) to reach the assimilatory tissues, the bark chlorenchyme is hidden behind dead peridermal or even rhytidomal multi layers. Thus, to efficiently drive photosynthesis, a sufficient amount of photosynthetically active radiation has to penetrate these layers to reach the light-harvesting complexes of the bark chloroplasts. Naturally, quality and quantity of light penetrating into a stem depends on the thickness, cellular structure and the optical properties of the outer bark layers (VOGELMANN 1993, PFANZ & ASCHAN 2000). Thus, it is obvious that the incident radiation is considerably weakened and subject to spectral changes before it reaches the chlorenchymal bark tissues. In *Betula pendula* the spectral composition of PAR is not equally reduced at all wavelengths, while passing the peridermal layers. In general, the longer the wavelength, and thus the lower the inherent energy, the better the light is transmitted through the peridermal tissues (Fig. 1c). Similar results are described by KAUPPI 1991 for *Betula pubescens* and *Betula pendula*. Our results show that (UV and) blue light is highly absorbed (Fig. 1a) in the peridermal layers whereas green and red light penetrates much further (see also KHAROUK & al. 1995, PFANZ & ASCHAN 2000). Thus, due to the selective peridermal absorption, the spectral light environment within the bark is clearly dominated by green and red light. The action spectrum of photo-inhibition shows that blue

light inhibits photosynthetic electron transport and thus oxygen evolution (EWART 1898, JONES & KOK 1966) more efficient than equivalent fluxes of red or green light. The low peridermal PFD transmission of blue light thus prevents photo-oxidation to a certain extent.

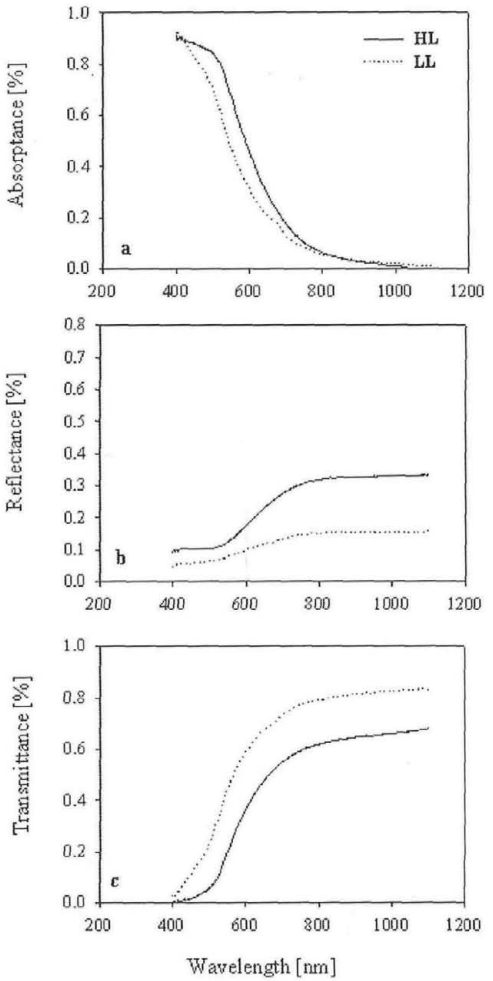

Fig. 1. Absorptance- (**a**), reflectance- (**b**) and transmittance spectra (**c**) of outer bark layers (periderm) between 400 and 1100 nm as measured on young birch twigs (1-year-old) grown at Low-Light (LL) or High-Light (HL).

Yet, also quantitative changes between incident PFD and PFD reaching the chlorenchyme were found. The light regime during growth clearly affected peridermal transmittance (Fig. 2 a, b). Under HL a marked reduction in the peridermal PFD transmittance of *Betula pendula* twigs was found; with increasing age, light was reduced from around 24% in young twigs (0- and 1-year-old) to around 10% in 3 (to 5)-year-old branches (Fig. 2a). Obviously this is due to the gradual thickening of the cork layers. Microscopy revealed the existence of an additionally formed, whitish peridermal layer in HL birches, a tissue not found in the LL-grown specimen. The additionally formed layer in the second year seems to be responsible for the described differences in PFD-transmission between HL- and

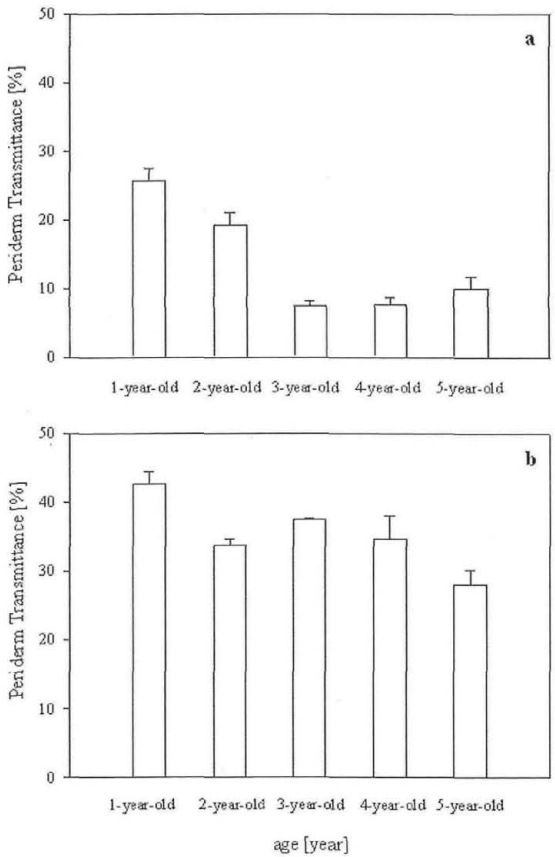

Fig. 2. Age-dependent peridermal transmittance [%] (between 400 and 700 nm) of peeled *Betula pendula* twigs (one- to five-year-old) grown under different light regimes (**a**: 100% of full sunlight; **b**: 20% of full sunlight) [means with SD, n=10].

LL-grown trees. BORGER & KOZLOWSKI 1972 showed that a stepwise increase in light intensity decreases the formation time of a first phellogen but increases the production of phellem in *Pinus resinosa, Fraxinus pennsylvanica*, and *Robinia pseudoacacia*. Trees exposed to HL therefore show more peridermal tissue than concomitant species growing under the forest canopy (ROMBERGER & al. 1993) and thus, have a lower peridermal light transmission.

Pigment content

On a unit surface area the chlorophyll contents of young birch twigs ranged from 99 to 166 mg Chl m^{-2} when trees were cultivated under HL and from 108 to 197 mg Chl m^{-2}, when trees were kept under LL (Table 1). Compared on a unit area basis the bark chlorenchymes in HL and LL grown trees contained between 47% and 49% of the chlorophyll of the concomitant leaves (Table 1). These data are in good agreement with those of SOLHAUG & al. 1995, PILARSKI 1984, KHAROUK & al. 1995 and SCHMIDT & al. 2000. In contrast to birch, this effect was less marked in the extremly shade-adapted species *Fagus sylvatica* where we observed chlorophyll contents of around 131 mg Chl m^{-2} in HL- and of 158 mg Chl m^{-2} in LL-grown trees (WITTMANN & al. 2001). Interestingly, under both light conditions a considerable increase of chlorophyll was found with increasing age of the birches (data not shown). It is well known that shade-leaves optimise the effectiveness of light absorption by an increased pigment density per unit leaf area and this strategy seems also to be true for the corresponding twig portions.

Table 1. Photosynthetic pigment content and chlorophyll a/b ratios of twigs and leaves of 6-year-old trees of *Betula pendula* cultivated under different light intensity regimes (100% and 20% sunlight). Measurements (n = 15-20) were performed in August 2002.

	Twig		Twig		Leaf	
Age	1-year-old		0-year-old		0-year-old	
Light intensity during growth [% sunlight]	100%	20%	100%	20%	100%	20%
Chl *a* [mg m^{-2}]	120 ± 19	134 ± 23	72 ± 7	75 ± 7	278 ± 39	311 ± 37
Chl *b* [mg m^{-2}]	46 ± 8	63 ± 9	27 ± 3	33 ± 4	76 ± 11	92 ± 17
Chl *a+b* [mg m^{-2}]	66 ± 26	197 ± 32	99 ± 10	108 ± 11	354 ± 50	402 ± 52
Chl *a/b*	2.6 ± 0.2	2.1 ± 0.1	2.7 ± 0.1	2.3 ± 0.2	3.7 ± 0.1	3.4 ± 0.3
Carotenoids [mg m^{-2}]	33 ± 13	24 ± 5	16 ± 2	13 ± 2	52 ± 5	33 ± 8
Chlorophyll/Carotenoids	6 ± 2	8 ± 1	6 ± 1	9 ± 2	7 ± 1	13 ± 6

Chlorophyll a/b ratios in birch bark ranged between 2.1 and 2.6. These values correspond nicely to those described for other deciduous trees, (*Fagus sylvatica* 1.8, LARCHER & al. 1988, *Populus tremuloides* 2.7, KHAROUK & al. 1995 and *Betula pendula* 2.5, KAUPPI 1991). The lower chlorophyll a/b ratios of the bark chlorenchyma (as compared to the leaves) are easily explained by the hidden location behind the cork layers.

In addition to chlorophyll, carotenoids serve at least two important functions in photosynthesis, namely light harvesting and photoprotection (LICHTENTHALER 1987, KOYAMA 1991, FRANK & COGDELL 1996). In HL-grown plants the main role of leaf carotenoids is protection of the photosynthetic apparatus from photo-oxidative damage. Accordingly, the HL-grown leaves showed a higher carotenoid pool size as compared to the LL-grown specimen (Table 1). In contrast, the area related carotenoid contents of the bark chlorenchymes (14 to 46 mg Car m^{-2}) showed no differences between the treatments. One explanation for this observation could be that in twigs and branches protective functions against photodamage and photoinhibition are primarily realized by the peridermal layers. Consequently, in bark chlorenchymes carotenoids act mainly as light-harvesting pigments by absorbing the transmitted sunlight and transferring the excitation energy to chlorophyll molecules. By this mechanism, photosynthetic organisms can utilize sunlight more efficiently, because carotenoids absorb green light which is abundant in sunlight but is weakly absorbed by chlorophyll (HAVAUX & al. 1998). The considerable increase of carotenoids with increasing age of the birch twigs (Table 1) can mainly be attributed to the intensified shading effect of the outer peridermal tissues and thus, an increased pressure for light-harvesting.

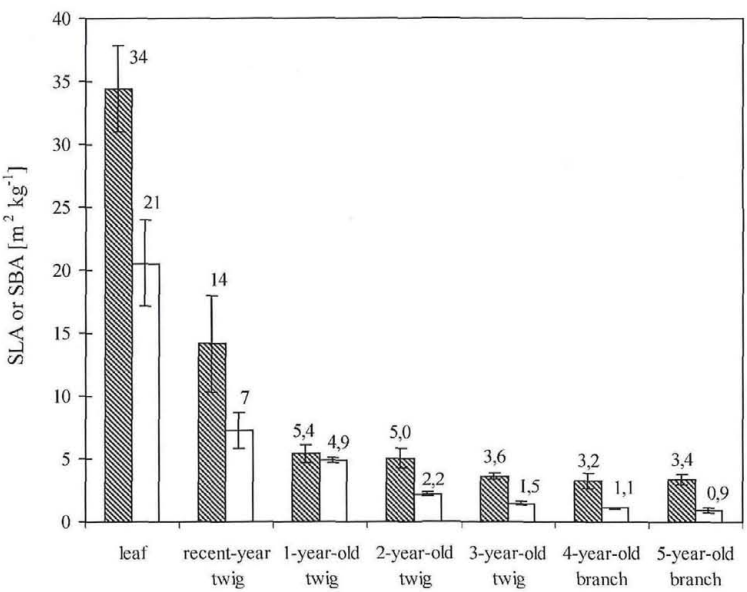

Fig. 3. Specific leaf area (SLA = projected leaf area per unit leaf dry matter) and specific bark area (SBA = projected bark area per unit bark dry matter) of leaves and young twigs of *Betula pendula* grown under different light regimes (20% of full sunlight: grey column; 100% of full sunlight: white column) [means +/- SD, n=10].

Specific leaf and bark area

The light environment has a large impact on leaf traits, especially on SLA (= the projected leaf area per unit dry matter (m^2 kg^{-1}); ELLSWORTH & REICH 1992). SLA is by definition related to the combination of leaf thickness and density and has been shown to correlate with either one or both (ABRAMS & al. 1994, GARNIER & LAURENT 1994). Plants grown in HL generally have thicker leaves and thus a low SLA; the increase in dry matter is partly due to extra layers or longer palisade cells (BJÖRKMANN 1981). Concomitantly, the number of chloroplasts and the amount of photosynthetic enzymes and thereby the photosynthetic capacity per unit leaf area is enhanced (EVANS & POORTER 2001). However, the increase in photosynthetic capacity of the HL-grown leaves comes at the cost of less light capture per unit biomass at lower irradiances. Consequently, birch plants grown under HL showed around 38% lower SLA values, as compared to the shaded variants and a similar tendency was also found at the bark level (Fig. 3).

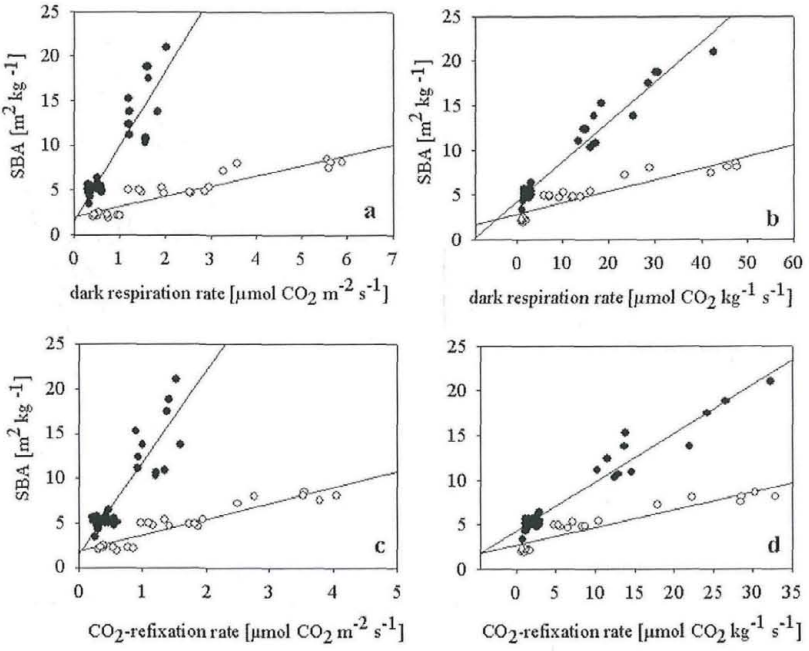

Fig. 4. The relationship between dark respiration (**a, b**), CO_2-refixation rate (**c, d**) and specific bark area in twigs (0y-2y) of young birch trees. (**b, d**): dark respiration rate and CO_2-refixation rate are converted to a mass basis. CO_2-exchange was measured at 1000 µmol PAR m^{-2} s^{-1} and bark surface temperatures of 20°C. (Low-Light: filled circles; High-Light: open circles). Correlation statistics are: (a): LL: f(x) = 8.38x + 1.57, R^2 = 0.83, P<0.0001, *n* = 32; HL: f(x) = 1.15x +2.06, R^2 = 0.85, P<0.0001, *n* =25. (b): LL: f(x) = 0.45x + 4.29, R^2 = 0.96, P<0.0001, *n* = 32; HL: f(x) = 0.13x + 2.84, R^2= 0.84, P<0.0001, *n* = 25. (c): LL: f(x) = 10.27x +1.45, R^2 = 0.81, P<0.0001, *n* = 32; HL: f(x) = 1.80x +1.81, R^2 = 0.87, P<0.0001, *n* = 25. (d): LL: f(x) = 0.55x + 4.26, R^2 = 0.95, P<0.0001, *n* = 32; HL: f(x) = 0.20x + 2.73, R^2 = 0.87, P<0.0001, *n* = 25.

The specific bark area (SBA, the projected bark area per unit bark dry matter), an integrated measure of bark thickness and density, showed a similar tendency; SBA of the LL-grown plants was up to 50% higher than in HL-grown specimen (Fig. 3).

It has been shown since long that variations in leaf structure (often quantified using SLA) lead to variations in photosynthesis and dark respiration (FIELD & MOONEY 1986, REICH & al. 1998); this relationship holds also true for the concomitant twig portions. Our results show that in addition to the leaves, the bark morphology, quantified as SBA, is significantly related to twig respiration (r^2 = 0.83-0.96) and CO_2-refixation rate (r^2 = 0.81-0.95) (Fig. 4). CERNUSAK & MARSHALL 2000 observed a correlation between specific bark area and CO_2-refixation rates in branches of western white pine. Variations in physiological and morphological traits among tree species have been related to differences in habitat conditions, including light availability (e.g. LOACH 1970, BAZZAZ 1979, WALTER & REICH 1996).

Gas exchange of twigs and leaves

When grown for two vegetation periods under different light regimes, young birches revealed typical photosynthetic light response curves for mature leaves and intact twig segments (Fig. 5). Compared to HL-grown birches, LL-grown leaves showed a considerable lower rate of maximum net photosynthesis of about 46% (14 to 8 µmol CO_2 m^{-2} s^{-1}; Fig. 5) and the light compensation point was reduced from 11 to 9 µmol m^{-2} s^{-1}. Additionally, LL-leaves revealed a decreased dark respiration rate (0.29 instead of 0.58 µmol CO_2 m^{-2} s^{-1}) and a lower saturating PFD of 700 µmol photons m^{-2} s^{-1} as compared to 1200 µmol photons m^{-2} s^{-1}. Besides the expected differences found between sun and shade grown leaves, also the physiological traits of birch twigs clearly differed between the treatments. In comparison to HL-grown trees, maximum net photosynthesis of LL twigs was increased by 30-40%, whereas apparent dark respiration was dramatically reduced (40-52%; Fig. 5). Also light saturation of birch twigs clearly differed between the treatments, being around 281-404 µmol photons m^{-2} s^{-1} in HL-grown twigs and 250-268 µmol photons m^{-2} s^{-1} in LL trees (Table 2). These results correspond to observations in a number of tree species (*Fagus crenata*, *Quercus acutissima;* HAN & SUZAKI 1981, *Populus tremuloides*, FOOTE & SCHAEDLE 1976b, *Acer rubrum,* COE & MCLAUGHLIN 1980, *Fagus sylvatica, Populus tremula,* WITTMANN & al. 2001); in all these species corticular photosynthesis saturated between 200 and 300 µmol PAR m^{-2} s^{-1}. According to LARCHER 2001, light saturation of deciduous shade leaves occurs around 200-500 µmol photons m^{-2} s^{-1}; corticular photosynthesis is thus performed by extremely shade adapted chloroplasts (which is also indicated by the low chlorophyll *a*/*b* ratio).

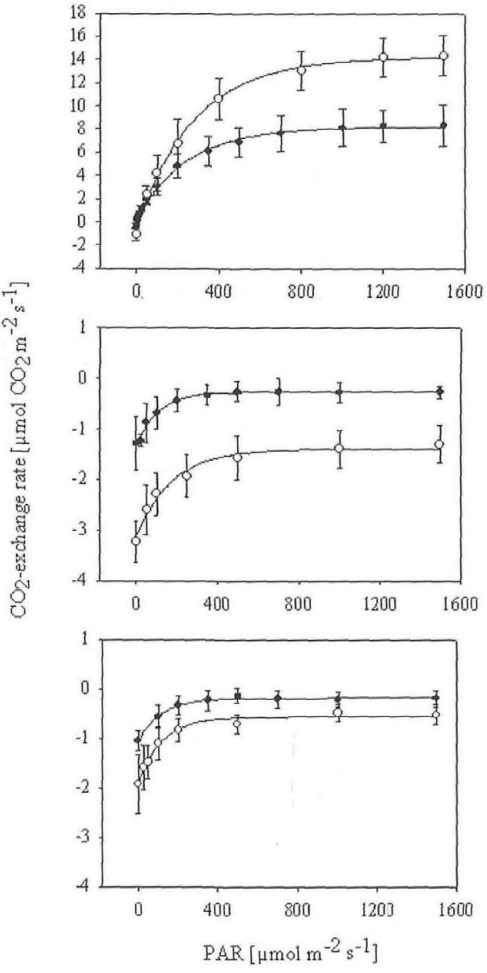

Fig. 5. Photosynthetic light response curves of *Betula pendula* leaves (upper panel) and twigs (middle and lower pannel) as measured under different light regimes (LL: filled circles; HL: open circles). Measurement were performed in June under constant climatic conditions (20% C, 50-55% relative humidity) and a controlled CO_2 supply (350 ppm) using a CO_2 porometer (means with SD, n=10).

Under neither treatment positive net photosynthesis was measured, but the CO_2-refixation rate and the relative CO_2-refixation (as expressed as percent of dark respiration) increased with light intensity (Fig. 6). Fitting of equation 1 to data from all current year twigs resulted in an average light response with a quantum efficiency of 0.0057 under HL and 0.0092 under LL, and a light-saturated CO_2-

refixation rate of 1.75 compared to 1.08 under shading (Table 2). Calculation of relative refixation, expressed as a percentage of dark respiration, revealed that under illumination on average 56% and 82% of the respired CO_2 was refixed within the twigs (Table 2). It is important to mention, that because of the high CO_2 concentrations in woody tissues, corticular CO_2-refixation is expected to proceed without photorespiration (1-26%: see McDOUGAL & WORKING 1933, CARRODUS & TRIFFETT 1975, EKLUND 1990, LEVY & al. 1999, PFANZ & al. 2002). FOOTE & SCHAEDLE 1976a assessed refixation ratios of about 85-92% in 6-year-old *Populus tremuloides*. Investigations in current- and one-year-old twigs of *Fagus sylvatica* and *Populus tremula* showed that corticular photosynthesis compensated the unavoidable CO_2-loss due to dark respiration by up to 90% (WITTMANN & al. 2001). Due to the higher proportion of living cortical and phloem parenchyma cells, refixation rates are generally higher in metabolically more active (i.e. younger) twigs than in older branches. But even at an age of 10 years, branches show a substantial photosynthetic activity with a relative refixation of 12% to 45% of apparent respiration (black alder: STEINBORN & al. 1997).

Table 2. Cardinal points of the light response curves of *Betula pendula* twigs grown under different light regimes (n = 10 ± SE). Maximal net photosynthesis (max. Ph(net)), max. CO_2-refixation rate (max. Ph(re)), saturated PFD (sat. PFD), dark respiration rate (Rd), relative refixation (rel. refixation) and photosynthetic quantum efficiency (α).

light intensity during growth [% sunlight]	20%	100%	20%	100%
Age of the stem organ	0-year-old		1-year-old	
max. Ph(net) [µmol CO_2 m^{-2} s^{-1}]	-0.26 ± 0.06	-1.39 ± 0.13	-0.19 ± 0.03	-0.56 ± 0.08
max. Ph(re) [µmol CO_2 m^{-2} s^{-1}]	1.08 ± 0.06	1.75 ± 0.13	0.87 ± 0.03	1.33 ± 0.08
sat. PFD [µmol photons $m^{-2}s^{-1}$]	250	404	268	281
Rd [µmol CO_2 $m^{-2}s^{-1}$]	-1.34 ± 0.05	-3.14 ± 0.11	-1.06 ± 0.03	-1.89 ± 0.07
rel. refixation [% of dark respiration]	81%	56%	82%	70%
α [µmol CO_2 $µmol^{-1}$ photons]	0.0092	0.0057	0.0086	0.0082

Light-saturated CO_2-refixation rates dropped within one year in both cultivation variants from 1.08-1.75 µmol m^{-2} s^{-1} to 0.87-1.33 µmol m^{-2} s^{-1} (Table 2) which was paralleled by a decrease in dark respiration; a strong correlation between CO_2-refixation- and dark respiration rate is often suggested (CERNUSAK & MARSHALL 2000, ASCHAN & al. 2001). Our results underline such a relationship also for birch twigs (Fig. 7a), where we found a striking relation between dark respiration of intact twig segments and their CO_2-refixation rate. The variability of dark respiration is responsible for 97% of the variation in CO_2-refixation rate (Fig. 7a; R^2 = 0.97, P<0.0001, n = 83; measured at 20° C and 1000 µmol PAR m^{-2} s^{-1}). The position and orientation of the (mostly) cylindrically shaped chlorenchyma

within the twigs and branches directly favours this relationship. The endogenous carbon dioxide that has been produced by woody-tissue respiration has to diffuse laterally out of the stem (if not dissolved in xylem water and transported to the heavily transpiring tree canopy, thus internally feeding leaf photosynthesis; MARTIN & al. 1994) and while passing the chlorenchymal barrier may be refixed during day-time.

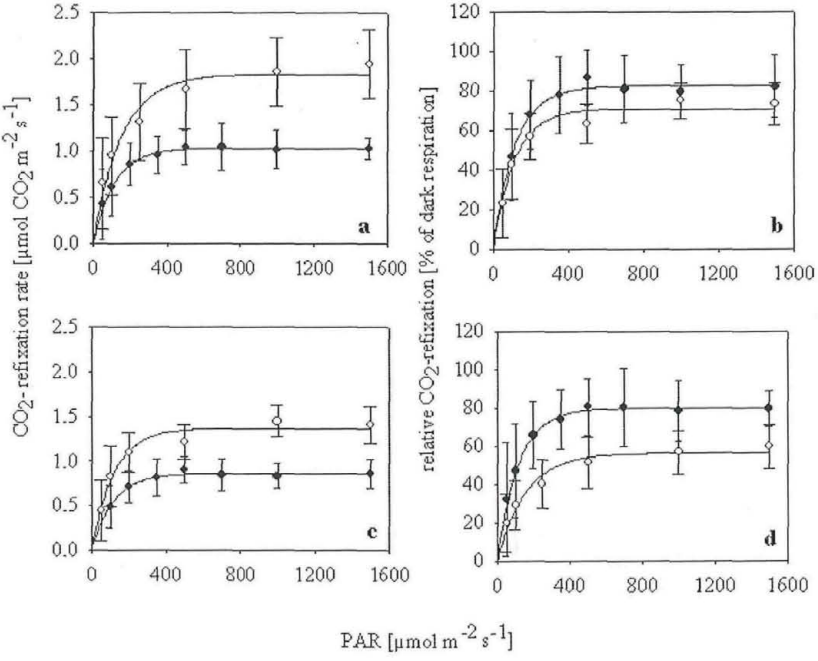

Fig. 6. The light response of current- (**a, b**) and one-year-old twigs (**c, d**) of *Betula pendula* expressed as (**a, c**) CO_2-refixation rate, calculated as the difference between dark and light respiration rates, and (**b, d**) refixation as a percentage of dark respiration. Measurements were made at twig surface temperatures of 20°C. Error bars represent SD; $n = 10$.

Variation in leaf dark respiration (R_d) has been widely proposed as a component of both, adaptation and acclimation to light availability. It has been argued that plants adapted to low light should have lower carbon losses via dark respiration; all species would then be expected to down-regulate R_d in shade, because the advantages of a high metabolic potential (high photosynthetic capacity) cannot be realized in such habitats (GRIME 1965, GIVINISH 1988, LUSK & REICH 2000). Our results show that besides leaf R_d also twig R_d is closely related to the light environment (Fig. 7a). Lower carbon losses in a twig could be achieved (1) by down-regulation of R_d or (2) by a higher relative CO_2-refixation via (up-regulation of)

corticular photosynthesis. Both pathways seem to be realised in young birch twigs (Fig. 7). The twigs showed a lower dark respiration but higher relative CO_2-refixation under shaded conditions; nearly 100% of the respired carbon were refixed in shaded trees (Fig. 6b).

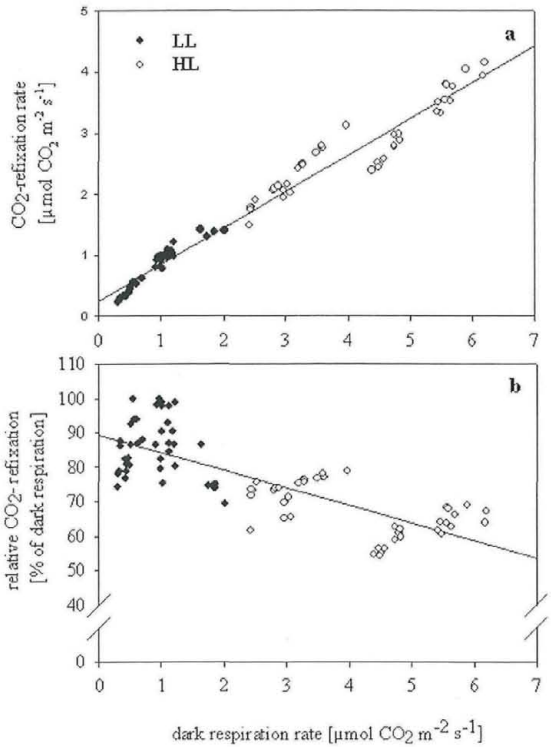

Fig. 7. The relationship between CO_2-refixation rate and dark respiration (a) [R^2=0.95, n=85] and between relative CO_2-refixation and dark respiration (b) [R^2=0.62, n=85] in current- to two-year old twigs of *Betula pendula*. Measurements were performed in July 2002 under constant climatic conditions (20°C, 50-55% relative humidity) and a controlled CO_2 supply (360 ppm) using a CO_2-porometer. (Low-Light: filled circles; High-Light: open circles).

The reason for the decrease in dark respiration with an increasing age of the twig organ is mainly the reduced ratio of living bark cells to (mainly) dead wood mass (NEGISI 1974) and also a generally higher resource turnover in younger plant parts. Therefore, the younger branches in outer parts of the canopy were found to have the highest respiration rates. As a consequence, the capacity for refixation of respiratory carbon was expected to be of greater importance in younger, still-growing and metabolically highly active parts of the crown and of minor im-

portance in the older stem fractions (see also WITTMANN & al. 2001). Yet, our results show that CO_2-refixation is not exclusively restricted to the light-exposed outer parts of tree crowns; relative refixation (Fig. 7) and light use efficiency of shaded inner twigs and branches can be clearly higher.

Conclusions

Our investigations showed that corticular CO_2-refixation is an important and overlooked component of the overall carbon balance of trees. Although twig and branch photosynthesis rarely results in positive net CO_2-fixation, a high portion of respired mitochondrial carbon dioxide is re-used within the tree skeleton and thus contributes substantially to the carbon balance and productivity of deciduous tress.

So far few studies have tried to integrate the stem-internal CO_2-refixation into a single tree or even stand carbon budget (for *Populus tremuloides* see FOOTE & SCHAEDLE 1976a, 1978). For young beech trees GANSERT 1994 found annual respiratory refixation rates of about 24%. KHAROUK & al. 1995 estimated the average bark input of *Populus tremuloides* as 10-15% during the midsummer vegetative period, and suggested that a larger, undetermined fraction may be held under environmental or phenological conditions where leaf photosynthesis is limited. Corticular CO_2-refixation may influence productivity up to stand level by increasing the carbon-use efficiency (CUE), the ratio of net primary production plus respiration. RYAN & al. 1997 estimated about 50% higher (above-ground) CUE for boreal stands dominated by *Populus tremuloides* than in conifer stands of *Picea* or *Pinus*. As a main reason for the offset in respiratory losses the effective recycling of respired CO_2 through refixation within the bark tissues is mentioned.

Yet, little is known about the mechanisms underlying the large variability in the respirational CO_2 loss from twigs and branches nor on the relationship between the CO_2 efflux and certain environmental parameters. As was shown for leaves, also bark morphology (e.g. SBA) clearly determines dark respiration (r^2 = 0.83-0.96) and corticular photosynthesis (r^2=0.81-0.95) and it thus can be used to predict the efflux of CO_2 from twigs. Furthermore, the striking correspondence between dark respiration of intact twig segments and CO_2-refixation rate leads us to suggest, that trees allocate their photosynthetic capacity for refixation relative to dark respiration rates. The latter being clearly related to the prevailing light environment. Similar observations were recently reported for the variation of the dark respiration of leaves (LUSK & REICH 2000).

Yet, to quantify the effects of corticular CO_2-refixation on the carbon balance of whole trees, exact morphometrical data on tree crowns are needed.

Acknowledgments

This paper is dedicated to Prof. Dr. Dieter GRILL on the occasion of his retirement. We would like to thank G. FRIESEWINKEL, S. KÜHR and Ch. KOSCH for technical assistance. Warm thanks also to Dipl. Umweltwiss. J. AHTING and M. HUMAR (Ljubljana). The discussions with Dr. G. ASCHAN are gratefully acknowledged.

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