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Photosynthetic Performance of Leaves and Twigs of Evergreen Holly (*Ilex aquifolium L.*)

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With 5 Figures

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Summary

SCHMIDT J., BATIC F. & PFANZ H. 2000. Photosynthetic performance of leaves and twigs of evergreen holly (*Ilex aquifolium* L.). – Phyton (Horn, Austria) 40 (1): 179–190, 5 figures. – English with German summary.

Twigs and stems of holly (*Ilex aquifolium*) contain up to 40 % of leaf chlorophyll (ca. 300 mg Chl. m^{-2} twig area) when compared on a unit surface area. Light penetration through the outer layers (epidermis, periderm or outer bark) of the shoot is age-dependent and ranges from roughly 40 % of transmitted sunlight in recent-year's twigs down to 5 % of rest light reaching the chlorophyll-containing chlorenchymous bark tissue in *Ilex* main stems. Oxygen gas exchange under optimum conditions revealed photosynthetic rates in mechanically isolated twig and branch chlorenchymes being nearly as high as comparable rates of leaves. Yet, high chlorenchymal photosynthesis was obtained only when tissues had been artificially separated. Intact tissues had much lower rates which can be explained by a highly impermeable *Ilex*-periderm and an effective stem- and twig-internal carbon cycling.

Zusammenfassung

SCHMIDT J., BATIC F. & PFANZ H. 2000. Photosyntheseleistung von Blättern und Zweigen der immergrünen Stechpalme (*Illex aquifolium* L.). – Phyton (Horn, Austria) 40 (1): 179–190, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Die Zweige und Stämme der Stechpalme (*Ilex aquifolium*) enthalten bei Oberflächenbezug bis zu 40 % des Chlorophylls von Blättern (ca. 300 mg Chl. m^{-2} Zweigoberfläche). Die Lichtpermeation der äußeren Schichten der Triebe (Epidermis, Borke oder äußere Rinde) ist altersabhängig und beläuft sich auf ungefähr 40 % transmittierten Sonnenlichtes bei diesjährigen Zweigen bzw. nur noch auf etwa 5 % Restlicht bei dem chlorophyllhaltigen Rindengewebe der älteren Hauptstämme der Stechpalme.

Unter optimalen Bedingungen zeigte der Sauerstoffgaswechsel mechanisch isolierter Zweig- und Astchlorenchyme Photosyntheseraten, die nahezu so hoch waren wie die der intakten *Ilex*-Blätter. Diese hohe Photosynthese des Chlorenchyms wurde allerdings nur bei künstlich abgetrennten Geweben erhalten. Intakte Gewebe wiesen deutlich geringere Raten auf. Dies wurde auf das Vorhandensein des stark undurchlässigen Periderms und einen effektiven, internen Kohlenstoffkreislauf innerhalb des Stammes und der Zweige (C-Recycling) zurückgeführt.

Introduction

Normal assimilatory organs are green; but also other plant parts such as stems or barks can be greenish too. The outer bark layers are brownish (oak) or grey (beech, aspen) or sometimes whitish (birch). However, on a closer look to bark tissues of younger twigs of trees one finds them regularly to be greenish. This clearly indicates the presence of chlorophyll-containing tissues. The chlorenchyma (as the chlorophyll-containing tissue is called) has been demonstrated to perform photosynthesis (FOOTE & SCHAEDLE 1976, LANGENFELD-HEYSER 1989, LARCHER & al. 1988, PILARSKI 1995, ZIEGLER 1957). Similar to photosynthesis in leaves, bark photosynthesis needs carbon dioxide and light as a prerequisite to drive photosynthesis. The gaseous substrate seems to be present in this tissue in reasonable concentrations. It has been published by MCDOUGAL & WORKING 1933 that up to 26,3 % CO_2 and sometimes even more were measured inside the living stems of trees (see also KAIPIAINEN & al. 1998, LEVY & al. 1999). Light is assumed to penetrate the outer dead bark parts (rhytidome) thereby being reduced in intesity to a certain extent. Also the water supply of the living bark cells (chlorenchyma) is thought to be mostly sufficient to allow metabolic action. We were interested in stem photosynthesis and wanted to know how effective bark photosynthesis can be performed in evergreen holly and under which circumstances it occurs.

Material and Methods

Plant material

80–100 year-old holly trees (*Ilex aquifolium* L.) from a south-east exposed forest edge were chosen for the studies. Leaves and shoot organs were collected and shoots were divided in three groups of different age. Group 1 consisted of obviously green twigs which were 1–2 years old and had a diameter of up to 0.6 cm; group 2 consisted of older branches with diameters between 0.8 to 2.0 cm. The third group of samples

was taken from the orthotropic main stem (diameter ca. 15 cm). Leaves were collected from extremely sun-exposed or from the extreme shade parts of the canopy. Samples were taken from June to September in the early morning hours shortly after sunrise. After transport to the laboratory in moist plastic bags, discs (∅ 5mm) were cut out from leaves or branches by the use of a cork borer. The high cambial activity of the sprout during the vegetation period also allowed bark and chlorenchyma peeling (RAO 1985, WORT 1962), which is a mechanically easy means of a nearly non-destructive separation of cortical layers from the wood fraction. When shoot diameter was too small, rectangular bark sections (approx. 1 cm^{-2}) were cut out with a razor blade. Gas exchange was measured (i) in isolated chlorenchyma, (ii) in peeled twigs with and without the adhering wood tissue, and (iii) in intact twig segments.

Chlorophyll determinations

30–70 mg fresh bark or peridermal tissue were sliced into 2mm sections and 1.5–3.0 ml 100 % DMSO (dimethylsulfoxide; with $Mg_2(OH)_2CO_3$) were added. As *Ilex* has strongly cutinized leaves and twigs, the material had to be ground beforehand to keep extraction time short. Chlorophyll was extracted for 2 h at 65 °C according to the method described in RONEN & GALUN 1984. $Mg_2(OH)_2CO_3$ was added to avoid acidification and a concomitant pheophytinisation of the chlorophylls. After spectrophotometry, the chlorophyll content was calculated using the equations of WELLBURN 1994.

Light penetration measurements

Light penetration was measured with the quantum radiometer LI 185A (LiCor, USA). The complete bark (chlorenchyma and rhytidome), or the mechanically separated dead rhytidome and the living chlorenchyma were used for the experiments. The samples were cut out from twig pieces with a cork borer (1.4 cm in diameter) and stored for 5min on isotonic solutions until use. White light was provided by a 100 W quartz halogen lamp (Osram Xenophot HLX 64625) equipped with an infrared filter (NIR filter ST 931619/KB, Balzers, Liechtenstein) to avoid warming of the samples. The circular samples were inserted into a black, self-made plastic clampholder which could be adjusted to tightly seal the sample margins by rubber O-rings (1cm in diameter) thus avoiding light penetration from the margins. The photosensor of the quantum meter was fixed directly below the sample. The light source was mounted above the sample holder and illumination was adjusted to yield a photon flux density (PPFD) of ca. 1000 $\mu E\ m^{-2}\ s^{-1}$. Different PPFDs were obtained by the use of neutral grey filters (Schott, Mainz, FRG). The irradiated and the transmitted light was recorded with and without the samples present and the absorbtion and transmission calculated.

Photosynthesis and respiration

Maximum photosynthetic capacity and respiration were measured as oxygen gas exchange (WALKER 1987) in thermostatically controlled, liquid-phase oxygen electrodes (Bachofer, Reutlingen, FRG) at 20 °C. The incubation medium consisted of 50 mM Mes (morpholino-ethane-sulfonic acid), 4 mM KCl, 1 mM $MgSO_4$, and 1 mM $Ca(NO_3)_2$, buffered at pH 6. Approximately 1 cm^2 fresh bark was cut from the stripes that had been peeled from the twigs and carefully infiltrated with incubation med-

ium. The pieces were placed in a 50 ml plastic syringe with 20 ml incubation medium (assay buffer) and all air was removed. A gentle vacuum was created by withdrawing the plunger. During the vacuum the syringe was agitated to dislodge any gas bubbles from the surface of the tissues. The vacuum was released and a slight positive pressure was applied (PFANZ & DIETZ 1987). The small bark pieces (0.5–1.0 cm^2) were suspended from the cuvette stopper by means of a small plasic-coated nichrome wire which was threaded through the injection hole. Illumination was provided by quartz halogen lamps. Before the actual measurement the tissues were pre-illuminated in the cuvettes for 60–70 mins. Calibrations and calculations were described by WALKER 1987.

Results and Discussion

Pigment content

When compared on a unit area basis, the mean chlorophyll content of holly leaves was 670 mg Chl m^{-2} projected leaf surface and thus only 2.5-times higher than the content of the axis organs (280 mg Chl m^{-2} axis surface) (Fig. 1). The differences between the Chl-contents of twigs, branches and stems were rather negligible. Compared to chlorophyll contents of leaves of other species the amount of twig chlorophyll is rather high (LAWLOR 1990). Being a shade plant, holly is expected to optimize the effectivity of light perception by an increased pigment density per unit leaf area (see LEVITT 1980). HEATH 1972 demonstrated that quantum flux exploitation of leaves is near 100 %, when chlorophyll density amounts to 250–300 mg Chl m^{-2} indicating that also young twigs of *Ilex* contain sufficient chlorophyll for a working photosynthesis.

Light penetration through the bark

When penetration of visible light through leaves was examined, only less than 1 % of the incident light hitting the upper epidermis was transmitted. More than 99 % were absorbed or reflected by the leaf tissue (Table 1). Those values are typical for thick or xeromorphic assimilation organs (LARCHER 1994). When peeled epidermal and peridermal twig layers were examined, nearly 40 % of the incident light were transmitted. Thus, under natural circumstances 40 % of the incident sun light can reach the chlorenchyma. The chlorenchyma itself is highly absorptive for visible light. Only 2.6 % of the incident light are not absorbed or reflected and can therefore reach the outer layers of the wood fraction of a twig. This proportion is even lower (0.2–1.0 %) when older twig and branch organs are examined (Table 1). But also the increasing thickness and the increasing in- and accrustation of polyphenols and lignins into the forming peridermal layers and dead-celled rhytidomes have a dramatic reductive effect in light penetration through the outer branch or stem tissues. Only 13.8 % or even only 5.5 % of the incident light are reaching the chlorenchyma of

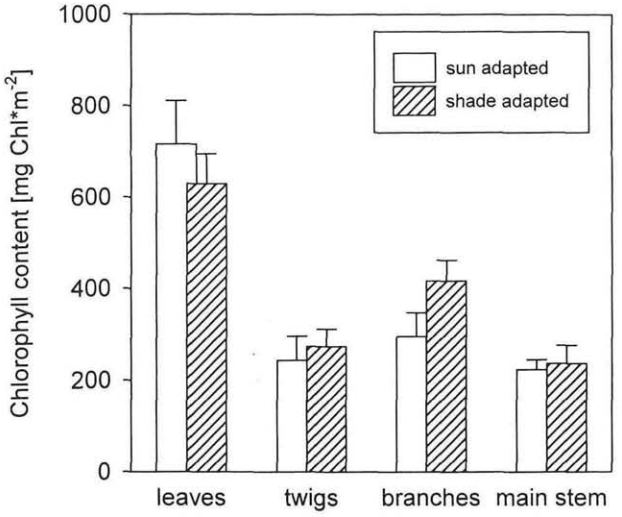

Fig. 1. Surface related chlorophyll contents of different organs of field-grown holly taken from fully sun-adapted or extremely shaded parts within the canopy (n=12).

older shoot parts. These results are in good agreement with those of KAUPPI 1991 and VOGELMAN 1993.

Table 1. Transmission of visible light through different green organs of holly (n=12). Transmitted light was calculated in percent of the incident light applied. Absorbed and reflected light were not differentiated.

Organ		Light transmission [%]
Leaves (intact)		0.7
Bark of twigs	Without chlorenchyma	38
	With chlorenchyma	2.6
Bark of branches	Without chlorenchyma	13.8
	With chlorenchyma	1.0
Bark of main stem	Without chlorenchyma	5.5
	With chlorenchyma	0.2

Photosynthetic gas exchange

Maximum photosynthetic capacity of the isolated chlorenchyma of axis organs measured under optimal conditions proofed to be extraordinarily high. Nearly 75 % of the rates of the surface-related carbon

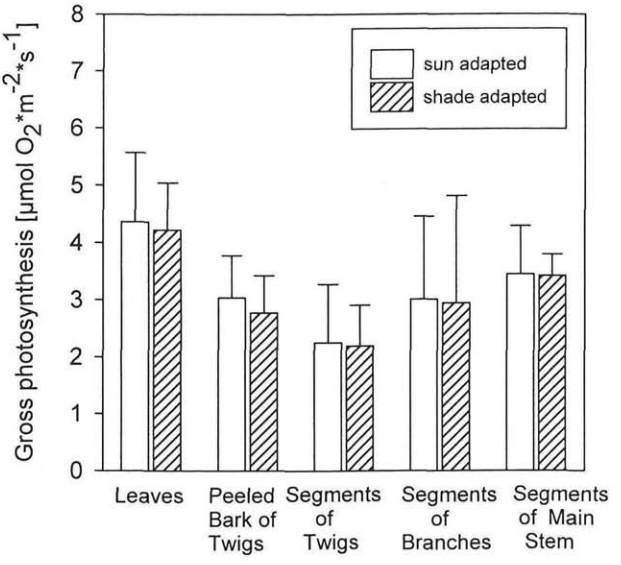

Fig. 2. Maximum gross photosynthesis of different organs of field-grown holly taken from fully sun-adapted or extremely shaded parts within the canopy. Peeled bark simply reflects the mechanically isolated outer dead bark and the living green chlorenchyma; in the case of twigs and branches, halved branches or twigs (bisected directly through the middle of the pith; length 1cm) were used. Stem photosynthesis was measured with circular sections (1cm in diameter) taken from the bark; the segments had been cut out by the use of a cork borer. Photosynthesis was measured under optimum conditions (light saturation at 400 µmol photons m^{-2} s^{-1}, CO_2 saturation at 3,5 mM $KHCO_3$ and a temperature of 20 °C) (n=24).

reduction rates of the concomitant leaves were obtained (Fig. 2). Not only twigs, but also branches and even the main stem revealed the ability to effectively photosynthesize at relatively high rates. When net photosynthesis of young twigs was measured, rates were remarkably higher when the heterotrophic wood fraction had been artifically removed (not shown). The reduced photosynthetic oxygen evolution of twigs with the wood fraction still attached is due to the high respiratory activity of the wood parenchymal cells (cf . BRAUNE & al. 1979, ESCHRICH 1995, SANDVED & al. 1993) (Fig. 3). Respiratory rates of young sun-adapted twigs were ca. 2.0 µmol O_2 $m^{-2}s^{-1}$ while the shade-adapted parts revealed slightly lower rates. Respiration rates became successively lower with increasing age of the twigs, which is thought to be a consequence of the lower fraction of living wood parenchymal or pith cells able to respire.

When photosynthesis was examined at an increasing light regime, only 250–300 µE m^{-2} s^{-1} PhAR were needed for obtaining maximum

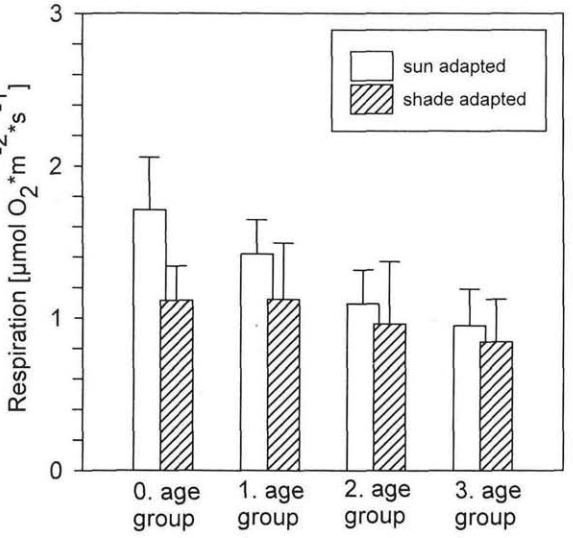

Fig. 3. Age dependend dark respiration of the wood fraction of twigs (0.–3. age group) of field-grown holly as measured in an oxygen electrode. 1cm long sections were used for all experiments (n=6).

rates, irrespective of whether leaves or peeled chlorenchymes from young twigs were used (Fig. 4). It has again to be mentioned, that holly is a typical shade tree. Furthermore, the comparison of photosynthesis of sun- and shade-adapted parts of holly showed no clear difference (Fig. 4). Chlorenchymal rates of net photosynthesis (1.2–2.0 µmol O_2 m^{-2} s^{-1}) were again half as high as in comparable leaves when measured under optimum conditions (2.5–3.0 µmol O_2 m^{-2} s^{-1}).

Photosynthesis also clearly decreased with an increasing age of the shoot (Fig. 5). Reduction of assimilatory carbon gain was observed although the respiratory carbon loss by the wood and pith parenchyma also decreased with increasing age (cf Fig. 3). A lowered light transmission through the rhytidome (dead outer bark), a decreased content in chlorophylls, and additional mechanisms (age-dependent?) seem to contribute to this behaviour. Radial segments of twigs (containing the respective part of wood and pith) revealed a photosynthetic performance of about 1.2–2.0 µmol O_2 m^{-2} s^{-1} whereas photosynthesis dropped in older branches to rates around 0.5 µmol O_2 m^{-2} s^{-1}. Although chlorophyll had been detected in the tree stems (cf Fig. 1) only respiration was measurable when stem chlorenchyma was illuminated. Nevertheless, compensation between respiratory CO_2 loss and assimilatory carbon gain was reached when the quantum flux density was higher than 300 µE m^{-2} s^{-1}.

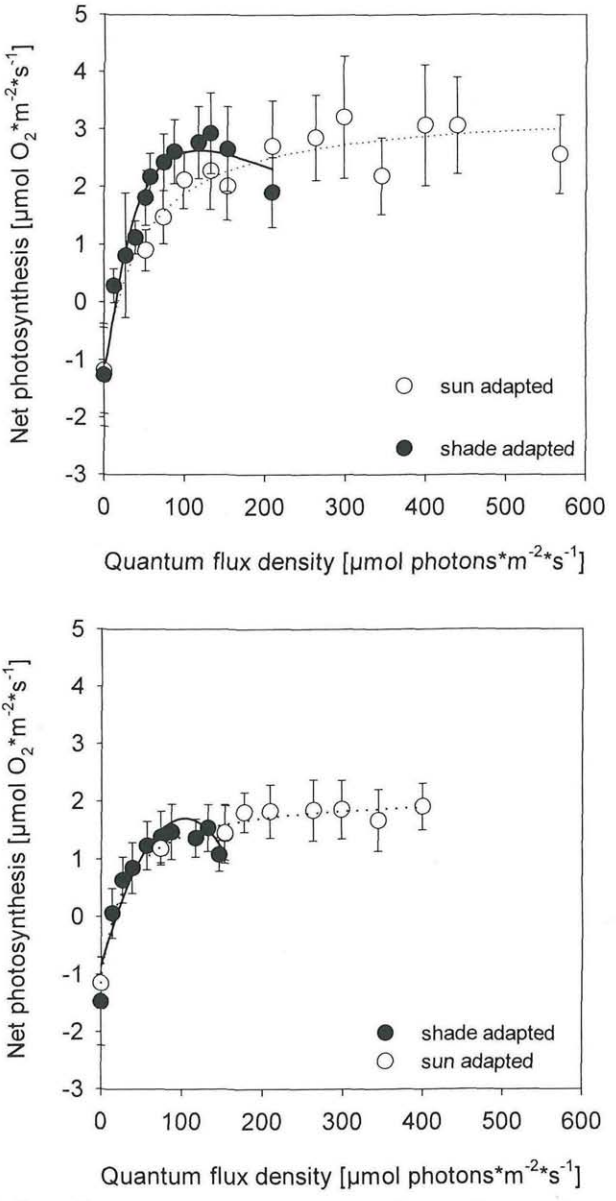

Fig. 4. Light dependencies of surface related net photosynthesis of leaves (0.–3. age group; left panel) and peeled bark of young twigs (0.–3. age group, right panel) as measured in an oxygen electrode under optimum conditions. The wood and pith fraction of the twigs had been carefully removed prior to the experiment. The open circles reflect samples taken from the sun-adapted parts of the canopy (solid circles = shade-adapted organs), (n=12).

Conclusions

Photosynthetic performance of the shoot of holly is not negligible (for other plants see SCHAEDLE 1975, PILARSKI 1995, NILSEN 1995, PFANZ & al. 1998, PFANZ 1999). It can be measured in young twigs, older branches and under higher light conditions even in stems. On a unit surface area, photosynthetic rates of isolated shoots fragments (isolated chlorenchyma) were up to 75 % of the respective leaves. A clear age dependency of stem photosynthesis is indicated by the decreasing rate of carbon reduction in older stem parts, which may be caused by the increasing thickness of the rhytidomal bark parts, thus absorbing too much incident light and the slight reduction of chlorenchymal chlorophyll. Also pith, wood and chlorenchymal respiration decrease with age as proportions shift to an increasing dead-wood-fraction in older parts of the shoots. It is not yet clear, whether bark photosynthesis is driven by external or more probable by internal CO_2 or even by both. The lack of stomates and the very low number of twig and branch lenticels indicate a very low CO_2-diffusion. Internal CO_2 re-fixation may act as an effective CO_2-recycling mechanism (cf also LEVY & al. 1999). In young oak trees (*Quercus phillyraeoides*) YIM & OGAWA 1969 found a stem area index SAI of 2.7 at a corresponding leaf area index LAI of 6.5. Although, in older trees this ratio may dramatically change, these numbers may give an idea of the stem area able to fix carbon dioxide.

Bark photosynthesis may therefore on the one side contribute to the overall carbon gain of holly throughout the year, and reduce carbon loss by internal CO_2-cycling. Stem photosynthesis may also temporarily compensate a loss of foliage caused by herbivorous insects, air pollution stress, or an attack of phytopathological fungi - questions that remain to be elucidated.

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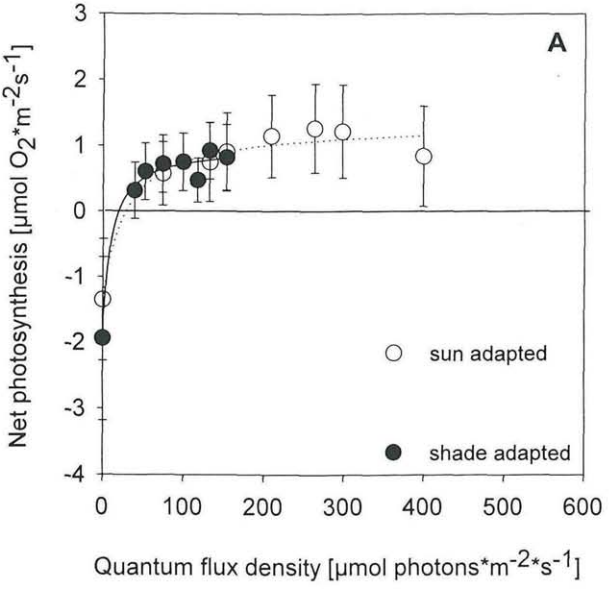

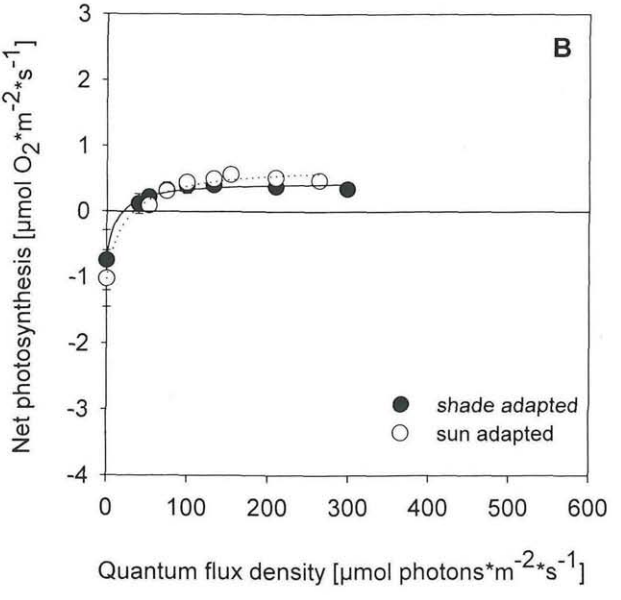

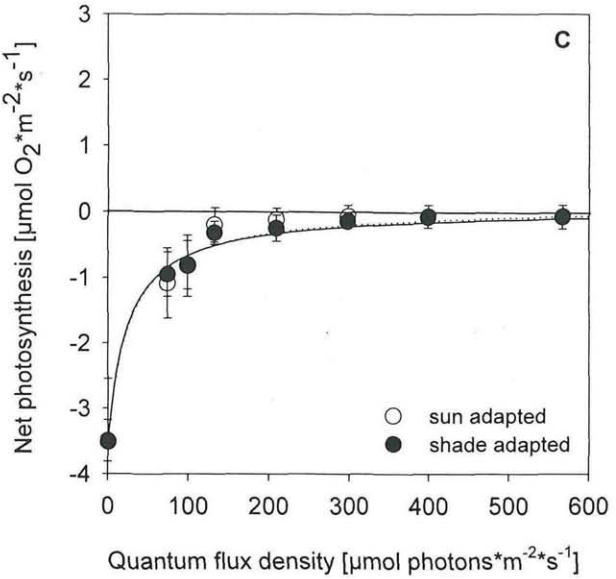

Fig. 5. Bark surface-area related net photosynthesis of intact segments of young twigs (0.–3. age group, A), older branches (bark plus rest of wood and pith fraction, B), and the main stem (bark plus rest of wood and pith fraction, C) as measured in an oxygen electrode under optimum conditions. The different tissues and fragments were identically taken and handled as decribed in the legend of Fig. 2. The open circles reflect samples taken from the sun-adapted parts of the canopy (solid circles = shade-adapted organs), (n=12).

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