

## Non-foliar photosynthesis and its contribution to the overall carbon balance of plants

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**Abstract.** In addition to the green leaves, commonly considered as the main sources of photosynthate production, higher plants can potentially use nearly all vegetative and reproductive structures to perform photosynthetic CO<sub>2</sub> assimilation. Green leaves, stems and green sterile flower organs, optimized for light harvesting and photosynthetic performance, are characterized by net photosynthetic assimilation utilizing mainly the atmospheric carbon dioxide. In contrast, chlorophyll-containing bark and wood tissue, most fruit, root and fertile flower organs are primary subordinated to non-photosynthetic functions, but typically perform an effective internal CO<sub>2</sub> recycling using the respiratory released CO<sub>2</sub>. Non-foliar photosynthesis, either manifested as positive net photosynthesis or internal CO<sub>2</sub> refixation is regarded as an important strategy of additional carbon acquisition. The main strategies of additional carbon acquisition by non-foliar chlorophyllous organs are illustrated, presenting selected examples developed in reproductive as well as in vegetative plant structures.

**Keywords:** floral photosynthesis, aerial roots, orchids, *Helleborus*, chlorophyll fluorescence

Abbreviations: Chl = chlorophyll

### Introduction

The visual impression of higher plants is typically dominated by green leaves as the main photosynthetic organ, but a closer look on herbaceous or even woody plants reveals a variety of non-foliar vegetative and moreover reproductive structures which contain chlorophyll and are thus potentially able to conduct photosynthetic CO<sub>2</sub> assimilation.

The positive net photosynthesis by green stems or flowers contrasts remarkably to the exclusive recycling of respiratory released CO<sub>2</sub> in fleshy fruits or bark tissues (BLANKE & LENZ 1989, PFANZ & AL. 2002). Independent of positive net photosynthesis or internal CO<sub>2</sub> refixation there is no doubt, that photosynthesis of chlorophyllous tissues other than leaf mesophyll will both partially pay for their own carbon requirements and thus positively contribute to the overall carbon gain of plants.

Since most existing information on the CO<sub>2</sub> exchange and carbon gain of higher vascular plants refers to leaves, data on the carbon budgets of stems, roots or fruits are almost entirely lacking (e.g. in epiphytes, see ZOTZ & HIETZ 2001). Therefore, we intend to illuminate the importance of non-

foliar photosynthesis as a strategy of additional carbon acquisition, presenting selected examples evolved in reproductive as well as in vegetative plant structures. While the photosynthetic activity of stem and bark tissues is mentioned elsewhere in this issue (see also PFANZ & ASCHAN 2001, PFANZ & AL. 2002), we are focussing here on representative case studies of floral and root photosynthesis.

## Methods

Carbon fixation in non-foliar plant organs has typically been measured using gas exchange or  $^{14}\text{C}$ -uptake techniques, but both methods may underestimate photosynthetic rates (e.g. BLANKE & LENZ 1989). Changes in external  $\text{CO}_2$  often not adequately reflect  $\text{CO}_2$  uptake in photosynthesis and in particular the amount of internally-generated photosynthetically fixed  $\text{CO}_2$  because of a low epidermal and cuticular permeability (cf. PFANZ & ASCHAN 2001). Especially on bulky green fruit, root or stem material, chlorophyll fluorescence techniques are advantageous, because photosynthetic performance doesn't depend on whether the  $\text{CO}_2$  fixed originates externally, or internally via respiratory processes (see SMILLIE 1992).

Chlorophyll fluorescence measurements on intact leaves and non-foliar organs were performed with a chlorophyll fluorometer PAM-210 (Walz, Effeltrich, Germany) under laboratory conditions (20°C).

Instant light-response curves of the effective quantum yield ( $Y = \Delta F/F_m'$ ) were obtained using the light-curve programme of the PAM (Run 10). After determination of maximum quantum yield a 5 min pre-illumination period at moderate light intensity (setting 3 corresponding to 120  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) provide a stationary fluorescence level of the samples. Then the actinic light intensity is decreased to setting 1 (60  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) for 5 min, before a saturation light pulse is applied to obtain Y and ETR. The apparent rate of photosynthetic electron transport of PSII (ETR) was calculated as  $\text{ETR} = \Delta F/F_m' \times \text{PFD} \times 0.5 \times 0.84$  (SCHREIBER & AL. 1995). Within the following 20 min light intensity is increased stepwise with illumination periods of 2 min and subsequent saturation pulses until setting 10 (1250  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) is reached.

*In situ* gas-exchange measurements on plant organs were conducted with a portable porometer system (LI-6400, Li-Cor Inc., Lincoln, USA). Photosynthetic light response curves were obtained under constant climatic conditions (20°C, 75 % relative humidity) and a controlled  $\text{CO}_2$  supply (350 ppm).

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

## Results and Discussion

### I. Carbon acquisition by reproductive structures

In the early developmental stages, petals of most flowering plants are green due to the presence of chlorophyll, whereas mature petals often are whitish or brightly coloured, because Chl is either absent or masked by other pigments. Therefore, photosynthetic activity was typically observed in visibly green petals (see DUECKER & ARDITTI 1968; VU & AL. 1985; SALOPEK-SONDI & AL. 2000), such as developed by the deciduous perennial Green Hellebore (*Helleborus viridis*). The photosynthetic light response of Green Hellebore sepals and leaves is studied by the comparative use of Chl

fluorescence as well as *in situ* gas-exchange measurements. The sepals achieved maximum electron transport rates of about 75 % of mature leaves at moderate to high PFDs, as determined by Chl fluorescence measurements (data not shown). In contrast, the maximum net CO<sub>2</sub>-exchange rates of Hellebore sepals (2.3 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) were less than one fourth of the leaves (10.6 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (Fig. 1). This difference is possibly explainable by the 70-80% lower stomatal density of the sepals in comparison to the Hellebore leaves.

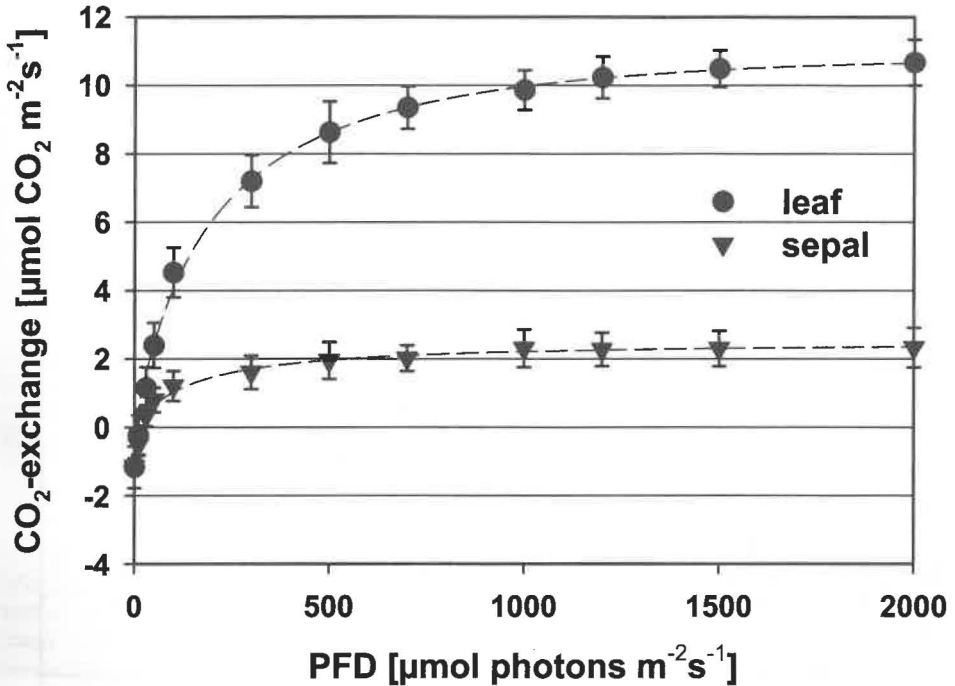


Fig. 1: Photosynthetic light response curves of Green Hellebore (*Helleborus viridis*) leaves (circles) and sepals (triangles). Measurements were performed in spring under constant climatic conditions (20°C, 75 % relative humidity) and a controlled CO<sub>2</sub> supply (350 ppm) using a CO<sub>2</sub>-porometer. Means with s.d. are presented; n = 12–14.

The sepals of the germane Christmas Rose (*Helleborus niger*) are whitish at anthesis and get gradually green (in shaded plants) or pinkish-red (in sun-exposed plants) during seed ripening. Maximum electron transport rates (ETR) of green sepals reached slightly lower values about 60% of the leaves' rates at medium PFDs (ca. 600 μmol photons m<sup>-2</sup> s<sup>-1</sup>), as assessed by Chl fluorescence measurements. The maximum electron transport rates of red sepals, coloured by anthocyan-like pigments, were clearly reduced to about one fourth of the respective leaf rates. As expected, *Helleborus* leaves use the quantum energy 1.5 fold more efficient than the green sepals, whereas the red ones need three times more quantum energy to transport the same amount of electrons within PSII (see ASCHAN & PFANZ 2003).

Using another method (O<sub>2</sub> gas-exchange) SALOPEK-SONDI & AL. (2000) reported photosynthetic capacities of greenish *Helleborus niger* sepals about 40–50 % of those of the green leaves. In greenish *Helleborus* sepals one third of the leaf Chl content (390 mg m<sup>-2</sup>, n = 6) was found, whereas red

sepals contained only about  $80 \text{ mg m}^{-2}$  Chl. Also for orchid bracts Chl contents about one third of the respective leaves were obtained (e.g. *Spiranthes cernua*: ANTLFINGER & WENDEL 1997), whereas those of the pure white petals only amounted to 4 %.

The pendulous, bell-shaped flowers of the Spring Snowflake (*Leucojum vernum*) have six equal white petals each tipped with an emerald green spot at the top. While the white areas of the petals are not photosynthetically active, even these small greenish spots achieve about 25 % of the ETR of the respective leaves (Fig. 2).

Remarkably, similar photosynthetic activity was obtained for the shorter inner petals of the white-flowering Snowdrop (*Galanthus nivalis*), which are also marked with a terminal green spot (data not shown).

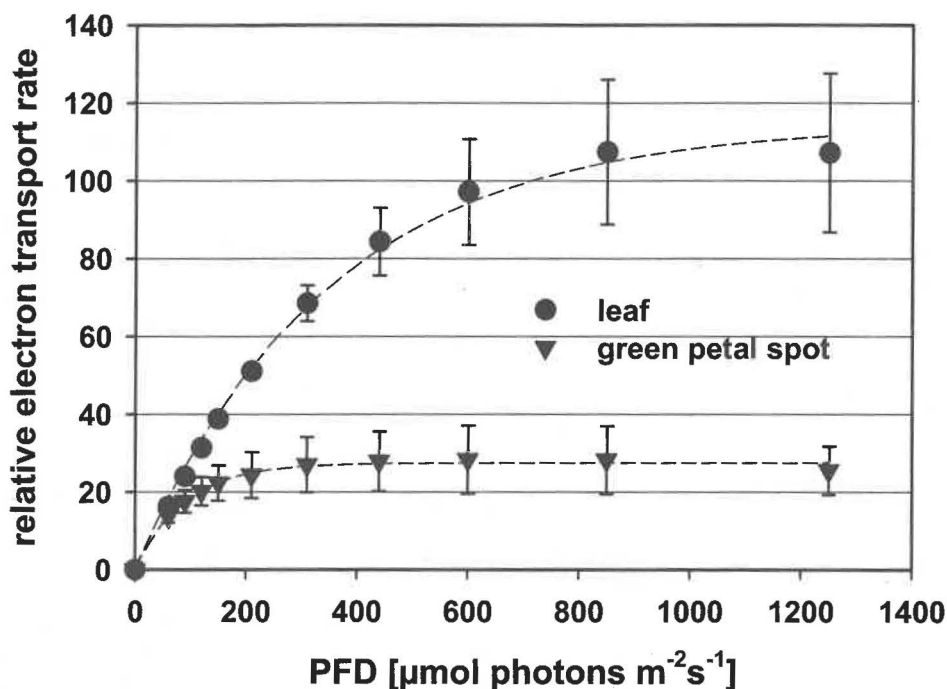


Fig. 2: Photosynthetic light response curves of leaves (circles) and green petal spots (triangles) of Spring snowflake (*Leucojum vernum*). Relative electron transport rate (ETR), calculated as  $\text{ETR} = 0.5 \times 0.84 \times \text{PFD} \times "F/F_m"$  (e.g. SCHREIBER ET AL. 1995), against photon flux density (PFD). Measurements were performed using a PAM-Fluorometer. Means with s.d. are presented;  $n = 9-15$ .

In general, photosynthetic rates of flowers vary widely between less than 1 to  $170 \text{ nmol CO}_2 \text{ g}^{-1} \text{ DW s}^{-1}$  or  $30-200 \mu\text{mol CO}_2 \text{ g}^{-1} \text{ Chl s}^{-1}$ , many perennials having lower values than those of annuals (see HEILMEIER & WHALE 1987). Even the  $\text{CO}_2$ -assimilation of *Lilium* anthers at  $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$  represents 73 % of the respective leaf  $\text{CO}_2$ -fixation (CLEMENT & AL. 1997a), whereas the  $\text{CO}_2$  fixation rate of green *Cymbidium* flower was only around 10 % of the leaf (DUEKER & ARDITTI 1968). Tab. 1 summarizes the photosynthetic rates of different green flowering plant species in comparison to the resp. leaf rates.

Table 1: Floral and leaf net photosynthetic rates of green flowering plants.

Species	Floral photosynthesis [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ]	Leaf	Author
<i>Helleborus viridis</i> (Ranunculaceae)	2.3 (sepals)	10.6	own measurements
<i>Aciphylla glaucescens</i> (Apiaceae): spines	11.0	13.5	HOGAN & AL. (1998)
<i>Helleborus niger</i> (Ranunculaceae)	295 (sepals)	592 – 741*	SALOPEK-SONDI & AL. (2000)
<i>Lilium hyb. enchantment</i> (Liliaceae)	2.3 (anther)1.8 (tepals)	3.2	CLEMENT & AL. (1997 a, b)
<i>Ranunculus adoneus</i> (Ranunculaceae)	-3.42 (young) -0.25 (fully expanded) 3.45 (petals abscised)	14.0–18.1	GALEN & AL. (1993)
<i>Spiranthes cernua</i> (Orchidaceae)	2.5 (flower), 3.7 (bud)	9.2	ANTLFINGER & WENDEL (1997)

\* in ( $\mu\text{mol O}_2 \text{ h}^{-1}\text{g}^{-1} \text{ DW}$ ).

Floral photosynthesis is considered to be an important additional source for assimilates within usually heterotrophic inflorescences and may thus provide a significant portion of the C requirement of reproduction. Reproductive structures could gain up to 60 % of their total carbon requirement, either acquired by floral or fruit photosynthesis (e.g. BAZZAZ & AL. 1979, WEISS & AL. 1988, MARCELIS & HOFMAN-EIJER 1995).

## II. Carbon acquisition by non-foliar vegetative organs

Non-foliar vegetative photosynthetic organs could represent the primary assimilate source, as often realized in stems (NILSEN 1995, PFANZ & ASCHAN 2001) and occasionally in roots, e.g. in leafless and nearly stemless orchid species (BENZING & AL. 1983, HEW & AL. 1984). Generally characterized by the lack of leaves as well as of stomates, the aerial roots of epiphytes (orchids, bromeliads, aroids) and vines, the green pneumatophores of mangroves or the stilt roots of some palms often contain chlorophyll and exhibit a well developed photosynthetic capability. In epiphytic orchids the chlorophyllous parenchymal tissues usually were masked by a spongy whitish layer, the *velamen radicum*, but the growing apex of the root is commonly green. Aerial root chlorophyll of some orchid species was found to range between 9 % and 55 % of respective leaf Chl contents, proportionately up to 89 % related to the fresh weight (ASCHAN & PFANZ 2003; see also BENZING & OTT 1981).

The roots of some orchid species are sufficiently autotrophic to either maintain themselves or contribute substantially to their needs (e.g. DYCUS & KNUDSON 1957). However, most green orchid roots (and stems) show no net photosynthesis (HEW & AL. 1984; BENZING & POCKMAN 1989), because of their stout structure, their lack of a regulated gas exchange and their predominant role for absorption and storage (BENZING & AL. 1983).

Regarding these functional properties of roots, the recycling of internally respired  $\text{CO}_2$  is considered as the main task of green, photosynthesizing root tissues. Preliminary gas-exchange measurements with aerial roots of two orchid species revealed  $\text{CO}_2$  refixation rates ranging from 49 % (*Doritis pulcherrima coerulea*) to 67 % (*Vanda spec.* data not shown), whereas no net photosynthesis was detected. In early gas-exchange experiments with the Warburg apparatus a reduction of respiratory  $\text{CO}_2$  evolution by aerial orchid roots is shown under high illumination (DYCUS & KNUDSON 1957).

Based on these data of four different orchid species we re-calculated root-internal  $\text{CO}_2$ -refixation rates between 40 and 50%.

Nevertheless, the photosynthetic light response of aerial orchid roots could be satisfactorily demonstrated using Chl fluorescence techniques. The chlorophyllous aerial roots of two selected orchid species achieved maximum electron transport rates between 55% (*Doritis pulcherrima coerulea*) and 65% (*Vanda spec.*) of mature leaves at PFDs between 600 and 1250  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . The aerial roots use the incident light energy only about 40% less effective than the orchid leaves, as shown by the slightly different initial slopes of the respective light curves (Fig. 3).

In spite of rather similar photosynthetic performance, the area-related Chl content of these two orchid roots differed considerably between 20% (*Doritis pulcherrima coerulea*) and 50% (*Vanda spec.*) of the respective leaf contents. Calculated on a unit Chl basis, electron transport rates in aerial roots thus exceed those of leaves.

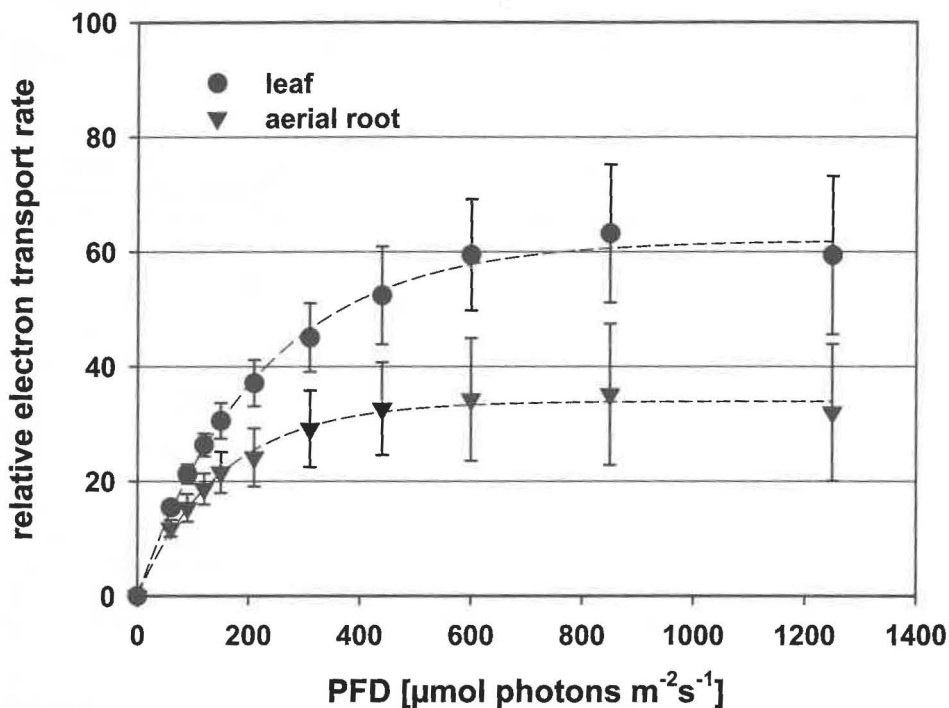


Fig. 3: Photosynthetic light response curves of leaves (circles) and aerial roots (triangles) of the orchid *Doritis pulcherrima coerulea*. Relative electron transport rate (ETR), calculated as  $\text{ETR} = 0.5 \times 0.84 \times \text{PFD} \times \text{''F/F}_m\text{'}$  (e.g. SCHREIBER ET AL. 1995), against photon flux density (PFD). Measurements were performed using a PAM-Fluorometer. Means with s.d. are presented; n=9-16.

A few leafless and (almost) stemless orchids photosynthesize only via flower spikes, stems (leafless *Vanilla*, leafless *Taeniophyllum*) or exclusively via green roots (e.g. the essentially shootless *Polyradicion*: BENZING ET AL. 1983; ghost orchid (*Polyrrhiza sp.*); *Microcoelia smithii*: DUEKER &

ARDITTI 1968; *Chiloschista usneoides*: COCKBURN ET AL. 1985). Such a strong reduction of the vegetative body is thought to enhance nutrient economy as an adaptation to extreme epiphytic life (BENZING & OTT 1981). Besides an evident refixation of respiratory CO<sub>2</sub>, the remaining green non-foliar organs obviously have to fulfil autotrophic functions.

### Concluding remarks

- Higher flowering plants could potentially use almost all vegetative and reproductive organs to perform photosynthetic CO<sub>2</sub> assimilation,
- non-foliar photosynthesis is either manifested as positive net photosynthesis, as demonstrated here for green flowers, or internal CO<sub>2</sub> refixation, typically found in chlorophyllous roots,
- green flowers or other photosynthetic parts associated with the inflorescence frequently conduct net photosynthetic CO<sub>2</sub> assimilation comparable to the leaves and thus may supply a significant fraction of the total carbon and energy costs of reproduction,
- aerial roots of orchids perform an efficient internal CO<sub>2</sub> recycling, compensating for up to two third of the respiratory released CO<sub>2</sub> and reaching electron transport rates about 60 % of the respective leaves.

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