Photosynthetic performance of vegetative and reproductive structures of green hellebore (*Helleborus viridis* L. agg.)

G. ASCHAN*, H. PFANZ*, D. VODNIK**, and F. BATIČ**

*Applied Botany, University of Duisburg-Essen, Universitätsstr. 5, D-45117 Essen, Germany**
*Agronomy Department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1001 Ljubljana, Slovenia***

Abstract

Photosynthetic irradiance response of vegetative and reproductive structures of the green-flowered deciduous perennial green hellebore was studied by the comparative use of chlorophyll (Chl) fluorescence techniques and gas exchange measurements. All the Chl-containing organs (leaves, sepals, stalks, and fruits) examined were photosynthetically active showing high intrinsic efficiencies of photosystem 2 (F_v/F_m: 0.75–0.79) after dark adaptation. Even in the smaller fertile and sterile parts of the flower (nectaries and anthers) a remarkable photosynthetic competence was detected. With increasing photon flux densities (PFD) electron transport rates, actual quantum yields, and photochemical quenching coefficients of the main photosynthetic organs decreased in the order: leaf>sepal>fruit>stalk. At moderate to high PFDs the sepals achieved maximum electron transport rates corresponding to about 80 % of concomitant mature leaves. In contrast, maximum net photosynthetic rate of the sepals [2.3 μmol(CO_2) m^{-2} s^{-1}] were less than one fourth of the leaves [10.6 μmol(CO_2) m^{-2} s^{-1}]. This difference is explained by a 70–80 % lower stomatal density of sepals in comparison to leaves. As the basal leaves emerge late during fruit development, the photosynthetically active sepals are a major source of assimilates, contributing more than 60 % of whole-plant CO_2 gain in early spring. The ripening dehiscent fruits are characterized by an effective internal re-fixation of the respirational carbon loss and thus additionally improve the overall carbon budget.

Additional key words: carbon budget; chlorophyll fluorescence; CO_2 re-fixation; floral photosynthesis; nectaries; sepals.

Introduction

Although leaves are traditionally considered as the main sources of photosynthates, also reproductive structures of many plants are photosynthetically active and therefore can fix substantial amounts of carbon (for a recent review see Aschan and Pfanz 2003a). Photosynthesis by reproductive structures has been observed in almost all sterile and fertile parts of the inflorescence, like bracts (*Arctium*: Heilmeier and Whale 1987; *Encelia*: Werk and Ehleringer 1983), sepals (*Cymbidium*: Dueker and Arditti 1968; *Paphiopedilum leeanum* hort.: Kirichenko *et al.* 1989; *Helleborus niger*: Salopek-Sondi *et al*. 2000), anthers (*Gasteria verrucosa*: Keijzer and Willemse 1988; *Lilium*: Clement *et al.* 1997a; cereals: Kirichenko *et al.* 1993), carpels (Galen *et al*. 1993), tepals and petals (*Lilium*: Clement *et al.* 1997b; *Petunia*: Weiss *et al*. 1988). Estimations of the photosynthetic contribution of green reproductive structures to their own carbon requirements vary and depend on the species, the type of organ, and its developmental phase, and on ambient conditions (Werk and Ehleringer 1983, Williams *et al*. 1985, Marcelis and Hofman-Eijer 1995). In some species, floral photosynthesis can provide a significant part of the carbon needed for reproduction (Bazzaz *et al.* 1979, Antlfinger and Wendel 1997). In the early developmental stages, petals of most flowering plants are green due to the presence of chlorophyll (Chl), whereas mature petals often are whitish or brightly coloured, because Chl is either absent or masked by other pigments (Weiss *et al*. 1988, Vainstein and Sharon 1993). Also the re-greening of brightly coloured parts of the perianth during seed development can be observed in several species, as the chromoplasts turn into functional chloroplasts (Grönegress 1974, Salopek-Sondi *et al*. 2002). Nevertheless, photosynthetic activity was typically observed in visibly green parts of the perianth, such as developed by the deciduous perennial green hellebore. The inflorescence buds of green hellebore develop very fast in the early spring and the green showy elements of the perianth commonly classified as sepals persist during seed ripening. Basal leaves of green hellebore emerge later in spring during fruit ripening, fully expand after seed maturity, and naturally die back in early winter (Werner and

Received 16 February 2004, *accepted* 23 September 2004.
*Fax. ++49-201-183-4219; e-mail: guido.aschan@uni-essen.de

Ebel 1994). Therefore, the Chl-containing sepals have to play an important role as a main source of assimilates for the developing fruits. A notable photosynthetic capacity was found in the re-greening sepals of the Christmas rose (Salopek-Sondi *et al*. 2000), but the contribution of floral photosynthesis to the plant carbon budget is not yet quantified. We compared photosynthetic activities in vegetative and reproductive structures of green hellebore grown in a natural forest environment and estimated the contribution of different plant parts to whole-plant carbon dioxide gain.

Materials and methods

Plants: In Italia and Slovenia the green-flowered hellebore species studied here is commonly determined as *Helleborus odorus* (Waldst. & Kit. in Willd.) or *H. viridis* L. var. *odorus* (Waldst. & Kit.) Fiori & Paol. Since status and differentiation of both hellebore species is controversial and not yet resolved, the more general denomination *Helleborus viridis* L. agg. is preferred. *H. viridis*, a deciduous perennial herb from the Ranunculaceae family, is naturally distributed within deciduous forests in the temperate to sub-mediterranean area, typically on calcareous soils (Werner and Ebel 1994). The green hellebore is 20–40 cm in height at flowering time (March–April), and the mature foliage consists of usually two basal leaves, pedately divided into 5–7 main segments, with the outer two again divided giving a total of up to about 7–13 divisions. Bracts subtending the branches and flowers vary in size and form. The few-flowered inflorescences include 2–4 wholly green flowers on long branches carried on slender pedicels, and the individual nodding flowers are 3–5 cm in diameter, regular in shape with their parts spirally arranged. The outer whorl consists of five large, usually overlapping perianth segments (sepals) which are yellow-green to intensely green, persisting through into the fruiting stage. The inner whorl (equivalent to petals) consists of 9–12 short-stalked, funnel shaped green nectaries. The fruit is made up of 3–4 several-seeded green follicles about 1.5–1.8 cm long at maturity, united at the base and dehiscent (Mathew 1989). In early March, whole plant individuals of *H. viridis* were dug out at its natural habitat in a deciduous forest (Hacquetio-Fagetum) at foothills of Šmarna gora close to Ljubljana, Slovenia, planted in plastic containers, filled with standard substrate, and carefully transported to the laboratory.

Photosynthetic pigments: Their contents in plant organs were assessed either from tissue discs removed by a calibrated cork borer (4 mm in diameter) or stem segments of known size and mass. Pigment extraction in dimethyl sulfoxide (DMSO) required approximately 2 h at 65 °C (Barnes *et al.* 1992). To avoid acidification and a concomitant phaeophytinisation of the Chls, 20 mg $Mg_2(OH)_2CO_3$ were added and extraction was conducted in the dark. Extract absorbances were measured with a spectrophotometer (*UV 160*, *Shimadzu*, Japan) and respective pigment contents were calculated according to equations of Wellburn (1994).

CO_2/H_2O exchange: *In situ* gas exchange measurements on intact plant organs of *H. viridis* were conducted with a portable photosynthesis system (*LI-6400*, *Li-Cor*, Lincoln, USA) in March and April. Dark respiration rate (R_D) was measured after a 30-min shading period of the respective plant parts within the cuvette. All gas-exchange measurements including subsequent PFD-response curves were carried out under constant climatic conditions (22–23 °C, 75 % relative humidity) and a controlled CO_2 supply (350 μmol mol^{-1}). Between 6 and 14 independent photosynthetic PFD-response curves were assessed for each type of plant organ. Curve fittings and calculations of all parameters were performed using *Sigma Plot 5.0* (*SPSS Science Software*).

O_2 exchange measurements on the rhizomatous rootstock of green hellebore were performed with thermostatically controlled (20 °C) liquid-phase Clark type electrodes (oxygen electrode unit *DW1*, *Hansatech*, UK) in standard incubation medium consisting of 50 mM MES, 4 mM KCl, 1 mM $MgSO_4$, and 1 mM $Ca(NO_3)_2$, buffered at pH 6.

Chl fluorescence of intact leaves and non-foliar organs was measured using pulse amplitude modulation fluorometer (*PAM-210*, *Walz*, Effeltrich, Germany) in the laboratory (20 °C). Due to their similar photosynthetic behaviour, bracts and basal leaves were not explicitly differentiated. Measurements were conducted on the adaxial (physiological upper) surface of the lamina of leaves and sepals. Instant PFD-response curves of the effective quantum yield ($Y = \Delta F/F_m'$) were obtained using the PFD-curve programme of the *PAM* (*Run 10*). After determination of maximum quantum yield in dark-adapted material (15 min) a 5-min pre-irradiation period at moderate irradiance [120 μmol(photon) m^{-2} s^{-1}] provided a stationary fluorescence level of the samples. Then the "actinic light" intensity was decreased to 60 μmol(photon) m^{-2} s^{-1} for 5 min, before a saturation pulse was applied to obtain yield and electron transport rate (ETR). Apparent rate of photosynthetic electron transport of photosystem 2 (PS2) was calculated as $ETR = \Delta F/F_m' \times PFD \times 0.5 \times 0.84$ (*e.g.* Schreiber *et al.* 1994). Within the following 20 min, irradiance was increased stepwise with irradiation periods of 2 min and subsequent saturation pulses until 1 250 μmol(photon) m^{-2} s^{-1} was reached. The photochemical quenching coefficient (q_P) was determined as $(F_m' - F)/(F_m' - F_0')$ and the non-photochemical quenching coefficient (q_N) as $(F_m - F_m')/(F_m - F_0')$ (Genty

et al. 1989). Additionally, photosynthetic competence of small reproductive structures such as green nectaries (petals) and anthers were examined using an Imaging *PAM* fluorometer (*Walz*, Effeltrich, Germany).

Biometrical assessments: Hellebore plants were harvested in early March and in late April; fresh mass (rhizome including roots, developing fruits) as well as the total surfaces of the individual plant organs (leaves, sepals, stalks including pedicels, peduncles, and petioles) were determined (area meter *LI-3100*, *Li-Cor*, USA). After drying to constant mass at 105 °C (≥24 h) dry masses were determined (balance *A 200 S*, *Sartorius*, Germany). Stomatal densities of all green organs were assessed at a mid magnification using light microscopy (*Aristoplan*, *Leitz*, Germany).

Results and discussion

Biomass: The inflorescences of the green hellebore usually develop very fast in early spring prior to the basal leaves that emerge later during fruit ripening; they fully expand after seed maturity. Regarding the total plant surface area (Fig. 1*A*, *upper panel*), in early spring the green hellebore is dominated by the sepals (56 %) and stalks (34 %), while the area of the few bracts amounts to only 10 % of total plant area. This proportion changes during growth and in late spring two-thirds of the total plant area is formed by basal leaves (including the bracts), 25 % by the stalks, and just 10 % by the sepals (Fig. 1*B*, *upper panel*). Because of the high surface-to-volume ratio half of the total dry matter is commonly represented by the stalks (Fig. 1*A,B*, *lower panels*). In early spring the remaining dry matter is distributed almost equally between the sepals (18 %) and the fruits (16 %), leaving 3 % for the bracts (Fig. 1*A*, *lower panel*). As the emerging basal leaves built up 40 % of total dry matter in late spring, the fraction of sepals is reduced to 6 % (Fig. 1*B*, *lower panel*). The mean dry mass per unit area of the flat photosynthetic organs leaf and sepal are much lower (45 or 40 g m^{-2}) than those of the stalks (94 g m^{-2}) and the dehiscent fruits (68 g m^{-2}). Regarding the dry mass, the root/shoot ratio of green hellebore in late spring was about 2.7±0.8 (n = 5). Based on a high dry mass per unit area of 198±18 g m^{-2} (n = 12), an average root/shoot area ratio of 1.2 was estimated.

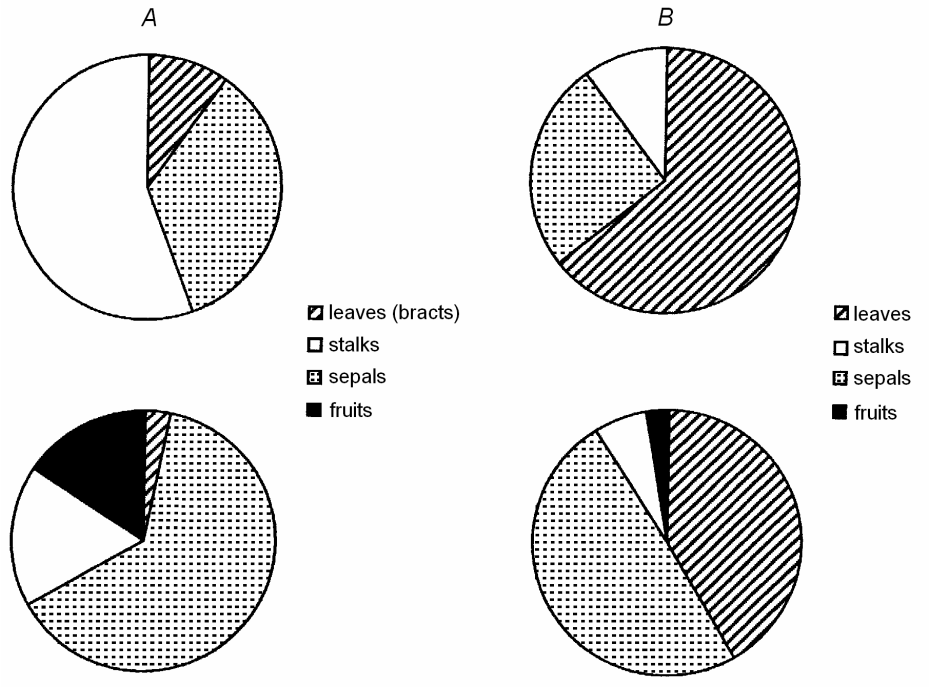

Fig. 1. Per cent contributions of the leaves, sepals, stalks, and fruits to the total surface area (*upper panels*) and dry matter (*lower panels*) of single green hellebore plants (n = 5) in (*A*) early and (*B*) late spring.

Stomatal density: To regulate the gas exchange of non-foliar organs, stomata similar to those of leaves are typically present in the epidermal layer of petals and sepals, but stomatal density is often lower than in leaves (see Aschan and Pfanz 2003a). In hellebore, stomata are abundant in leaves, sepals, fruits, and stalks with a decreasing density: leaves 111±24 mm^{-2}, sepals 23±3 mm^{-2}, fruits 22±10 mm^{-2}, stalks 19±9 mm (n = 20–30). On sepals of nine orchid species 1 to 6 stomata mm^{-2} were assessed, whereas respective leaves were covered with 34–151 sto-

mata mm^{-2} (Hew *et al.* 1980). Higher maximum stomatal densities of about 200 stomata mm^{-2} are found on sepals of apple, while respective leaves have on average 270 stomata mm^{-2} (Vemmos and Goldwin 1993). In floral bracts of tropical orchids stomatal densities even are comparable to the green leaves (Antlfinger and Wendel 1997). The epidermal layers of *Citrus* fruits contain stomata at a 3–10 times lower frequency than the epidermises of the respective leaves (Moreshet and Green 1980).

Pigment content: The presence of Chl in all hellebore tissues indicates that not only leaves but also sepals, stalks, and fruits are potential assimilatory organs, able to perform photosynthesis. On a unit surface area, total Chl contents were significantly lower in ripening fruits and stalks than in green leaves, whereas green sepals included about 50 % of the total mature leaf Chl (Table 1). In re-greened sepals of *Helleborus niger* one third of the leaf Chl content (130 resp. 390 mg m^{-2} was found, whereas dark red sepals contained only about 80 mg m^{-2} Chl (Aschan and Pfanz 2003b). Also on a fresh mass basis, mature leaves of *H. niger* comprised three to four times higher Chl contents than green sepals (Salopek-Sondi *et al*. 2000). For orchid bracts, Chl contents of about one third of the respective leaves were obtained (*e.g. Spiranthes cernua*: Antlfinger and Wendel 1997), whereas those of white petals only amounted to 4 %. Maximum Chl contents in *Lilium* tepals represented 22 % of the leaf Chl content (Clement *et al.* 1997a). Even in mature non-green corollas of *Petunia* Chl contents up to 40 % of the respective leaves could be found (Weiss *et al.* 1988). The sepals of apple flowers contain more than half of the rosette leaf Chl (124–221 respectively 251–407 mg m^{-2}; Vemmos and Goldwin 1993). The highest Chl *a/b* ratios were noted for the hellebore fruits (3.1), while the ratios of the remaining organs varied slightly between 2.5 and 2.7. An increase in Chl *b* is thought to maximize photon harvesting and the relatively low ratios may reflect a response to the shaded forest understorey where the plant material was collected. Chl *a/b* ratios about 2.6 were found in the re-greening sepals of *H. niger* (Salopek-Sondi *et al*. 2000), too. The Chl *a/b* ratios of apple flower parts, petioles, and peduncles lay in the range 2.8–3.2 (Vemmos and Goldwin 1993). Chl *a/b* ratios in green *Dendrobium* plant parts (2.6–2.8) were generally higher than in developing flowers (sepals: 2.3–2.6; petals: 1.7–2.2; Khoo *et al.* 1997).

Table 1. Chlorophyll (Chl) content (mean±SD, n = 8-12) and Chl fluorescence characteristics (maximum electron transport rate, ETR; photochemical efficiency, F_v/F_m; quantum yield; photochemical quenching, q_P; non-photochemical quenching, q_N) in different plant parts of green hellebore (leaf, sepal, stalk, fruit). All values are for an "actinic light" of 850 μmol(photon) m^{-2} s^{-1}. F_v/F_m was determined after a dark adaption for 15 min. Means±SD (n = 12–48). *T*-tests were performed for pairs of corresponding values (leaf *vs*. sepal, stalk, fruit); significantly different values are indicated (non significant $p>0.05$, $^{*}0.05>p>0.01$, $^{**}0.010>p>0.001$, $^{***}p<0.001$).

	Leaf	Sepal	Stalk	Fruit
Chlorophyll content [mg m^{-2}]	383±61	184±69***	72±21***	92±14***
Chl *a/b*	2.7±0.3	2.6±0.3 n.s.	2.5±0.3 n.s.	3.1±0.8 n.s.
Maximum ETR	121±13	97±11***	76±11***	98±15***
F_v/F_m	0.77±0.04	0.78±0.02 n.s.	0.75±0.03 n.s.	0.79±0.02*
Quantum yield	0.34±0.04	0.27±0.04***	0.21±0.03***	0.27±0.04***
q_P	0.77±0.12	0.68±0.13**	0.48±0.13***	0.51±0.10***
q_N	0.72±0.14	0.83±0.07***	0.75±0.09 n.s.	0.71±0.05 n.s.

Chl fluorescence analysis: Photochemical activity of the different green plant parts was verified by Chl fluorescence techniques. The F_v/F_m ratio indicates the intrinsic efficiency of PS2 photochemistry in the absence of light. This ratio differed only insignificantly in the plant parts examined varying between 0.75 (stalk) and 0.79 (fruit, see Table 1), which is slightly lower than the average of the values found in leaves of a wide range of C_3 species (0.83: Björkman and Demmig 1987). The relatively high values found in green hellebore demonstrate that all of the green organs exert a high efficiency of photon utilization in PS2. Similar F_v/F_m ratios were described for sepals (0.71), fruits, or stalks (0.79) of the orchid *Dendrobium* (Khoo *et al.* 1997) and for sepals, fruits (0.78), or petioles (0.77) of tomato plants (Hetherington *et al.* 1998). Photosynthetic electron transport rates (ETR) in stalks, fruits, sepals, leaves, nectaries (petals), and anthers of green hellebore were determined as a function of PFD (Figs. 2*A* and 3*A*). All Chl-containing organs considered were photosynthetically active with ETR showing an almost linear increase up to PFDs of 500 μmol(photon) m^{-2} s^{-1} and thereafter rising more slowly to reach a plateau at higher PFD. However, distinct differences existed between the different organs both in maximal ETR and PFD necessary for photon-saturated rates. The sepals and the ripening fruits achieved maximum ETR of about 80 % of mature leaves at moderate to high PFDs, whereas the stalks only reached about 60 % (Table 1). Somewhat lower maximum ETR values were assessed for the nectaries and anthers (50±6 or 34±4), too. The trend towards a lower ETR in stalks, nectaries, and anthers usually indicates a more shade-type photosynthesis, which is explainable by the vertical orientation or hidden position of these plant parts. For

ripening mango fruits electron transport rates between 93 and 117 were described (Hetherington 1997), whereas in different parts of tomato plants higher maximal ETRs were found (fruit: 110; sepal: 170; stalk tissues: 180–250; leaf: >300; Hetherington *et al*. 1998). Effective quantum yield of all green plant parts generally decreased with rising PFD (Figs. 2*B* and 3*B*). The leaves are characterized by the highest quantum yields, followed by the sepals and fruits with 20 % lower but similar values, while the stalks showed the greatest decline in actual efficiency of PS2 at high PFDs (Table 1). In comparison, PS2 quantum yield of *Petunia* corolla was 20 % lower than in the respective leaf (Weiss *et al.* 1988).

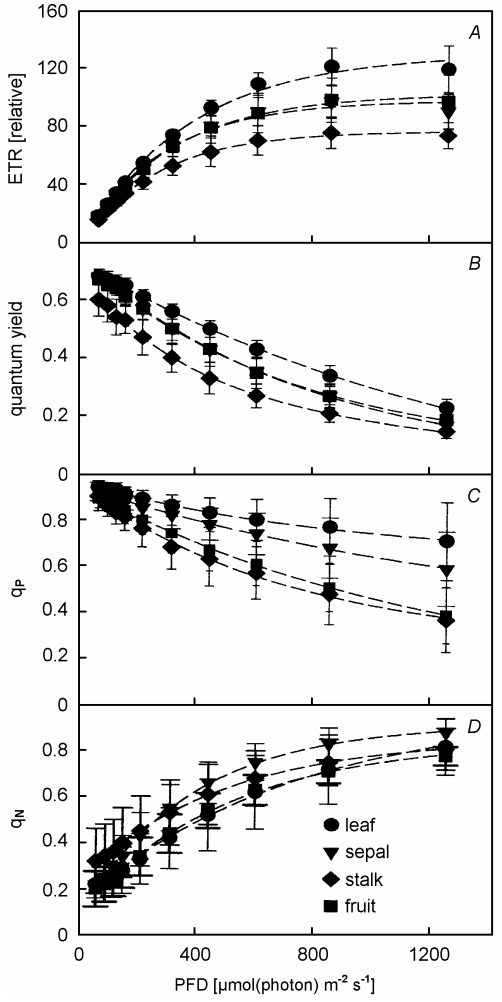

Fig. 2. Photosynthetic photon flux density (PFD) response curves of leaves, sepals, stalks, and fruits of *Helleborus viridis*. (*A*) Relative electron transport rate (ETR), calculated as ETR = 0.5 × 0.84 × PFD × $\Delta F/F_m'$. (*B*) Quantum yield of photosystem 2 as assessed by the fluorescence parameter Y = $\Delta F/F_m'$. (*C*) Photochemical (q_P) and (*D*) non-photochemical (q_N) quenching coefficients. Means±SD. *n* = 12–48].

Chl fluorescence quenching analysis provides additional information on the photosynthetic responses of the different plant parts to changing irradiance. The allocation of absorbed photon energy to photochemical and non-photochemical pathways is indicated by the quenching coefficients. The photochemical quenching coefficient q_P represents a measure of the oxidative state of the primary acceptor of PS2, Q_A. Initially oxidized at zero PFD, and thus having the maximum potential for photochemical activity, an increasing proportion of the acceptor becomes reduced as PFD is increased, and correspondingly the value of q_P decreases below 1. Generally, the more shade acclimated the tissue, the larger the decrease in q_P at high PFD. It is shown in Fig. 2*C* that in leaves the acceptor of PS2 was maintained in a more oxidized state at a given PFD than in the sepals, fruits, and stalks. At PFD of 850 µmol(photon) m^{-2} s^{-1}, the PS2 acceptor was reduced to about 80 % (leaf), 70 % (sepal), and less than 50 % (fruit and stalk) of the initial dark-adapted state, proving that fruits and stalks are less efficient in the utilization of photon energy than leaves and sepals of green hellebore. The non-photochemical quenching coefficient q_N reflects the energy dissipation of PS2. A general increase of q_N was observed in all plant parts with rising PFD (Fig. 2*D*). The increase in q_N in the sepals was higher than that of the leaf, stalks, and fruits, showing small differences at higher PFD. However, the significant higher q_N of the sepals suggested a more effective thermal dissipation of excessive radiant energy than in the other green organs. This might be an advantage in early spring protecting the sepals during a period

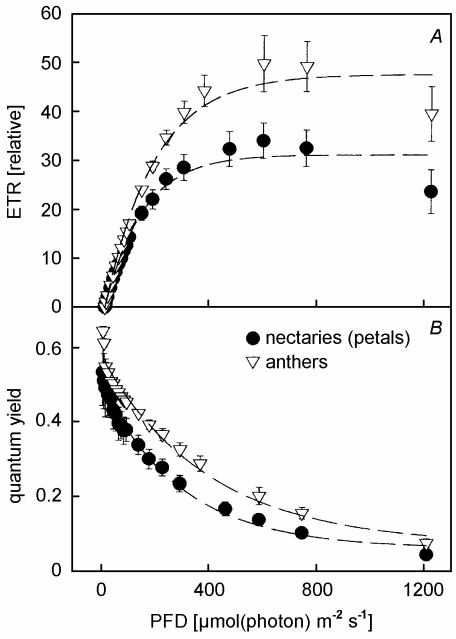

Fig. 3. Photosynthetic photon flux density (PFD) response curves of nectaries (petals) and anthers of *Helleborus viridis*. (*A*) Relative electron transport rate (ETR), calculated as ETR = 0.5 × 0.84 × PFD × $\Delta F/F_m'$. (*B*) Quantum yield of photosystem 2 as assessed by the fluorescence parameter Y = $\Delta F/F_m'$. Means±SD. *n* = 12–24].

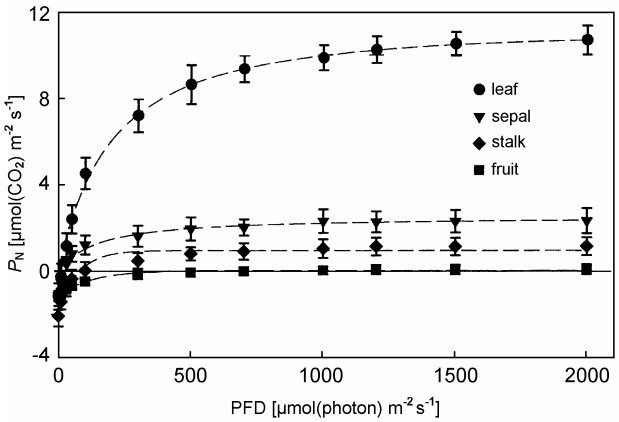

Fig. 4. Photosynthetic photon flux density (PFD) response curves of leaves including bracts, sepals, stalks, and fruits of *Helleborus viridis*. Measured in spring under constant climatic conditions (22–23 °C, 75 % relative humidity) and controlled CO_2 supply (350 g m^{-3}) using a CO_2-porometer. Means±SD. n = 6–14.

of high irradiance that frequently occurs in the leafless season of a deciduous forest habitat.

CO_2 exchange: In parallel to fluorescence techniques, CO_2/H_2O gas exchange of the different organs was investigated. The leaves, sepals, and stalks showed net CO_2 assimilation with increasing PFD (Fig. 4), whereas ripening fruits were only able to compensate the respiratory CO_2 release up to a slight net uptake. Leaves generally showed the highest P_N and the most effective quantum use efficiency; they furthermore had the lowest compensation irradiance of all green organs. Mean maximum P_N of hellebore sepals [2.3 µmol(CO_2) m^{-2} s^{-1}] was less than one fourth of the mature leaves [10.6 µmol(CO_2) m^{-2} s^{-1}], but R_D was similar (Table 2). Compensation irradiances between 10 and 20 µmol(photon) m^{-2} s^{-1} as well as maximum photosynthetic rates between 10 and 15 µmol(CO_2) m^{-2} s^{-1} are considered as typical in leaves of early-flowering geophytes (see Larcher 2001). Under elevated CO_2 concentration (2 000 µmol mol^{-1}) the photosynthetic capacities of the sepals were enhanced to almost 50 % of the respective leaf capacities. Using O_2 gas-exchange measurements, Salopek-Sondi *et al*. (2000) assessed resembling photosynthetic capacities of re-greened sepals of *H. niger* of about 25–50 % of the concomitant green leaves. Young inflorescences of the terrestrial orchid *Spiranthes cernua* achieved one third of the leaf photosynthesis (Antlfinger and Wendel 1997), whereas the CO_2 fixation rate of green *Cymbidium* flowers amounted to only 10 % of that of the leaf (Dueker and Arditti 1968). On an area basis, the stalks reached about half of the sepal P_N, whereas the fruits possessed only a slight P_N under high PFD and very low quantum use efficiency. The stalks revealed the highest R_D of all organs, they needed substantial PFD to reach the compensation irradiance, and their quantum use efficiency was comparable to that of sepals. Considering the greater surface to dry matter ratio of the stalks, its P_N expressed per unit dry mass only amounted to 4 % of the leaves and 17 % of the sepals (Table 2). P_N of sepals were in the lower part of the broad range, documented for flower photosynthesis in the literature [1–170 µmol(CO_2) kg^{-1}(DM) s^{-1}) (see, *e.g.*, Heilmeier and Whale 1987). For the rhizomatous rootstock mean R_D of about –0.46±0.18 µmol(O_2) m^{-2} s^{-1} was detected using liquid-phase oxygen electrodes (n = 12).

Table 2. Values for cardinal points of PFD-response curves in different plant parts of green hellebore (n = 6-14). Means±SD. n.d. = not determined.

		Leaf	Sepal	Stalk	Fruit
Dark respiration	[µmol(CO_2) m^{-2} s^{-1}]	–1.2±0.6	–1.1±0.2	–2.0±0.5	–1.2±0.1
Compensation irradiance	[µmol(photon) m^{-2} s^{-1}]	15	24	80	495
Max. net photosynthetic rate	[µmol(CO_2) m^{-2} s^{-1}]	10.6±0.5/23.5±2.1	2.3±0.5/11.4±1.8	1.1±0.4/n.d.	0.1±0.2/n.d.
	350/2 000 µmol mol^{-1}(CO_2)				
	[mmol(CO_2) kg^{-1}(Chl) s^{-1}]	28	13	15	1
	[mmol(CO_2) kg^{-1}(DM) s^{-1}]	0.23	0.06	0.01	0.001
Quantum use efficiency	[mol(CO_2) mol^{-1}(photon)]	0.07	0.05	0.04	<0.01

On a Chl basis, average maximum P_N of *H. viridis* leaves was two times higher than that of sepals and stalks, whereas in the leaves and sepals of *H. niger* almost identical photosynthetic capacities were detected (measured as oxygen exchange in aqueous solution; Salopek-Sondi *et al*. 2000). But taking into account the 5-fold lower stomatal density of the sepals and 10-fold lower values of the stalks, the "real" photosynthetic assimilatory capacity of those green plant organs could be higher. As reported in several studies, green parts of reproductive structures have a 1–3 times higher photosynthetic assimilatory capacity than the leaves of the same plant species (Luthra *et al.* 1983, Werk and Ehleringer 1983, Williams *et al.* 1985, Heilmeier and Whale 1987, Smillie 1992). A compensation of the respirational CO_2 loss up to a marginal apparent net photosynthesis of the dehiscent hellebore fruits is typical for a variety of green fruits including fleshy fruits (apple, grape berry), indehiscent fruits (cereal grain), and dehiscent fruits (pea pods), which commonly re-fix much internally respired carbon but

only modest amounts of atmospheric CO_2 mainly during early development (Blanke and Lenz 1989, Aschan and Pfanz 2003a).

Stomata in orchid flowers are considered as "non-functional", failing to respond to irradiance, VPD, CO_2 concentration, and abscisic acid (Hew *et al.* 1980). Due to a continuous CO_2 release during day and night, a permanent and at least partial stomata opening is assumed (Hew *et al.* 1980, Goh 1983). For the hellebore sepals low stomatal conductances (g_s) were observed also in the dark suggesting a partial stomata opening. In contrast, the sepals showed a clear positive relationship between PFD and g_s ($r^2 = 0.77–0.99$, $n = 14$ independent PFD curves). With increasing VPD a decrease in g_s of sepals was evident, accompanied by a linear increase in transpiration up to maximum rates of 1.0–1.5 mmol m^{-2} s^{-1} (Fig. 5) representing about half of the respective leaf transpiration (data not shown). A similar function of stomata in the control of transpiration was found in young inflorescences of *S. cernua*, whereas mature inflorescences showed no significant difference between dark and light values of g_s (Antlfinger and Wendel 1997).

For whole plants, P_N typically represents the rate of acquisition of carbon and energy. Based on the single PFD-response curves (Fig. 4) and the mean surface areas of each Chl-containing organ (Fig. 1) the relative photosynthetic contribution of the different parts to the total plant carbon dioxide exchange was estimated by multiplying the given rate per area times the total area of the

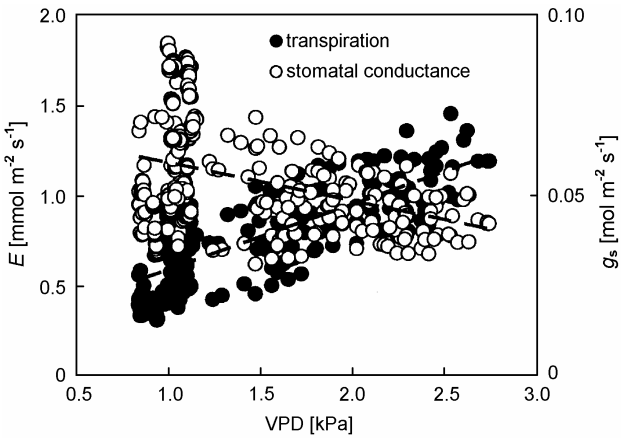

Fig. 5. Vapour pressure deficit (VDP) dependency of transpiration rate (E) and stomatal conductance (g_s) of hellebore sepals measured under saturating PFD [≥1 000 µmol(photon) m^{-2} s^{-1}], mean air temperatures about 24±2 °C, and controlled CO_2 supply (350 g m^{-3}) using a CO_2-porometer.

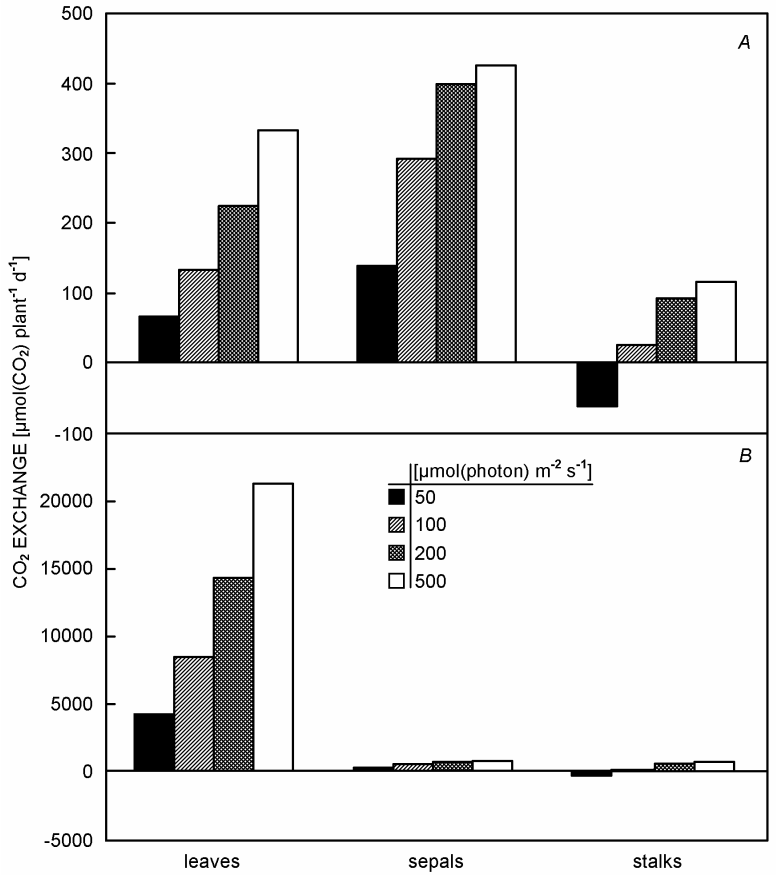

Fig. 6. Total daily CO_2 exchange of the leaves, sepals, and stalks of an intact green hellebore plant as estimated under different irradiances [mean PFD: 50, 100, 200, 500 µmol(photon) m^{-2} s^{-1}] in (*A*) early spring and (*B*) late spring.

respective organ. As a first approximation, we assumed an identical PFD supply for the entire hellebore plant neglecting the different orientations and shapes of the various plant components and also an inevitable temperature response of photosynthesis.

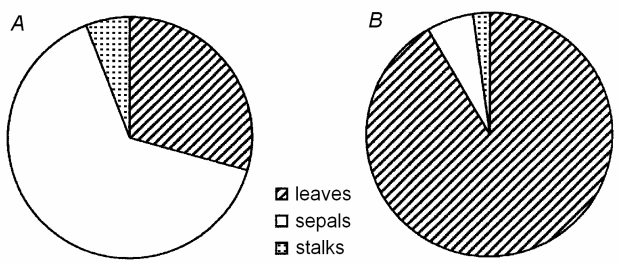

Fig. 7. Per cent contribution of the main photosynthetic parts (leaf, sepal, stalk) of green hellebore to the whole-plant carbon dioxide gain in (*A*) early spring and (*B*) late spring; a mean PFD of 100 μmol(photon) m^{-2} s^{-1} is assumed.

Assuming a day-length of 10 h and optimal irradiance, a small-sized developing green hellebore plant could assimilate about 1 mmol(CO_2) d^{-1} in early spring. However, in a natural hellebore habitat, *e.g.* a deciduous forest understorey, low irradiances usually prevail, but taking into account a higher photon input during leafless state in early spring (Aschan and Lösch 2000), a mean PFD of 50 or 100 μmol(photon) m^{-2} s^{-1} giving a daily total of 1.8 or 3.6 mol(photon) m^{-2} d^{-1} seems to be more realistic. Simply regarding the aboveground biomass, a net CO_2 uptake of a young hellebore plant reached at PDF of about 30 μmol(photon) m^{-2} s^{-1}. With a mean PFD of 100 μmol m^{-2} s^{-1} a total daily CO_2 gain of 0.5 mmol(CO_2) d^{-1} is calculated (Fig. 6*A*). Under all PFD regimes the green sepals are the main assimilate source contributing up to two-thirds of total daily CO_2 gain in early spring, while the few bracts contributed by another 30 % (Fig. 7*A*). Whereas the green stalks respire under low irradiance, their CO_2 uptake was enhanced to more than 10 % of total carbon gain under higher PFDs. In contrast to the early stage, aboveground CO_2 gain of fully developed hellebore plants is enhanced markedly to more than 20 mmol(CO_2) d^{-1} in late spring (Fig. 6*B*). About 90 % of the total daily carbon gain is then provided by the mature basal leaves and a couple of bracts. The contribution of the sepals is reduced to 6 %, the remaining part is acquired by the stalks (Fig. 7*B*). As a first approximation, a total daily carbon loss of about 2 mmol(CO_2) d^{-1} was estimated for the belowground parts of fully developed hellebores, which is compensated by photosynthetic carbon uptake of the aboveground parts already under mean PFD of about 30 μmol(photon) m^{-2} s^{-1}. Assuming a comparable R_D for the rootstock also in early spring, the small young plant without mature basal leaves was not able to compensate the respiratory carbon loss even under high PFD. The resources stored in the rhizomatous rootstock have to be allocated either to vegetative growth or reproduction. Carbon assimilation of green sepals thus compensates to some extent for energy costs associated with the production of seeds. The carbon amount supplied by photosynthesis of flowers to their own carbon budget typically differs between 8.4 % (*Spiranthes cernua*: Antlfinger and Wendel 1997), 15 or 30 % (*Arctium tomentosum, A. lappa*: Heilmeier and Whale 1987), 33 % (*Malus pumila*: Vemmos and Goldwin 1994), 41–57 % (*Ambrosia trifida*: Bazzaz and Carlson 1979) up to 64.5 % (*Acer platanoides:* Bazzaz *et al*. 1979).

Conclusions: Photosynthesis of reproductive structures is a prominent mechanism enabling compensation of the costs of reproduction in plants (Obeso 2002). In many plant species, the additional carbon amount supplied from floral photosynthesis is regarded as an important contribution to the total carbon required for maintenance, nectar allocation, and the production of mature seeds. The carbon skeletons and the inherent energy assimilated by reproductive structures are predominately consumed for their own growth and development (Vemmos and Goldwin 1994). The carbon fraction provided by reproductive structures generally varies in a broad range between less than one and more than 60 % of their total carbon requirement (Bazzaz *et al.* 1979, Watson and Caspar 1984). However, a significant proportion of the total energy budget must be supplied by belowground structures.

In early spring, the young fruits of green hellebore are ripening when last year leaves are dying and the new generation of basal leaves is just expanding. The specific sink for the carbon assimilated by the hellebore inflorescence cannot be exactly predicted from the data collected, but the unfolding leaves are expected to spend most of the assimilates for their own growth. Therefore, despite of the few bracts the Chl-containing sepals are confirmed as the main assimilate source in early spring achieving a net CO_2 uptake for the aboveground plant, especially under the prevailing irradiances in their natural forest habitat. In general, the temporary season for carbon gain is extended in winter-deciduous plants by the photosynthetic activity of such flower parts. Another developmental advantage is assumed for the green sepals even during bud stage because of the irradiated position as the outermost whorl protecting and feeding the enclosed reproductive structures. Also in the orchid *Dendrobium* the sepals were considered as the major flower part responsible for photosynthesis at all developmental stages (Khoo *et al*. 1997).

Due to the remarkable photosynthetic activity of the green sepals and the effective CO_2 re-fixation of the ripening fruits a significant contribution of the reproductive structures to their total carbon need could be verified for *H. viridis*. Such adaptations to improve plant carbon balance may lower the costs of reproduction in green hellebore and facilitate frequent seed production.

References

Antlfinger, A.E., Wendel, L.F.: Reproductive effort and floral photosynthesis in *Spiranthes cernua* (Orchidaceae). – Amer. J. Bot. **84**: 769-780, 1997.

Aschan, G., Lösch, R.: Das Bestandesklima niederbergischer Buchenwälder. – Jahresber. naturwiss. Verein. Wuppertal **53**: 89-111, 2000.

Aschan, G., Pfanz, H.: Non-foliar photosynthesis – a strategy of additional carbon acquisition. – Flora **198**: 81-97, 2003a.

Aschan, G., Pfanz, H.: Non-foliar photosynthesis and its contribution to the overall carbon balance of plants. – Acta biol. sloven. **46**(2): 3-10, 2003b.

Bazzaz, F.A., Carlson, R.W.: Photosynthetic contribution of flowers and seeds to reproductive effort of an annual colonizer. – New Phytol. **82**: 223-232, 1979.

Bazzaz, F.A., Carlson, R.W., Harper, J.L.: Contribution to reproductive effort by photosynthesis of flowers and fruits. – Nature **279**: 554-555, 1979.

Blanke, M.M., Lenz, F.: Fruit photosynthesis. – Plant Cell Environ. **12**: 31-46, 1989.

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

Clement, C., Mischler, P., Burrus, M., Audran, J.C.: Characteristics of the photosynthetic apparatus and CO_2-fixation in the flower bud of *Lilium*. I. Corolla. – Int. J. Plant Sci. **158**: 794-800, 1997a.

Clement, C., Mischler, P., Burrus, M., Audran, J.C.: Characteristics of the photosynthetic apparatus and CO_2-fixation in the flower bud of *Lilium*. II. Anther. – Int. J. Plant Sci. **158**: 801-810, 1997b.

Dueker, J., Arditti, J.: Photosynthetic $^{14}CO_2$ fixation by green *Cymbidium* (Orchidaceae) flowers. – Plant Physiol. **43**: 130-132, 1968.

Galen, C., Dawson, T.E., Stanton, M.L.: Carpels as leaves: meeting the carbon cost of reproduction in an alpine buttercup. – Oecologia **95**: 187-193, 1993.

Genty, B.E., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Goh, C.J.: Rhythms of acidity and CO_2 production in orchid flowers. – New Phytol. **93**: 25-32, 1983.

Grönegress, P.: The structure of chromoplasts and their conversion to chloroplasts. – J. Microscopie (Paris) **19:** 183-192, 1974.

Heilmeier, H., Whale, D.M.: Carbon dioxide assimilation in the flowerhead of *Arctium.* – Oecologia **73**: 109-115, 1987.

Hetherington, S.E.: Profiling photosynthetic competence in mango fruit. – J. hortic. Sci. **72**: 755-763, 1997.

Hetherington, S.E., Smillie, R.M., Davies, W.J.: Photosynthetic activities of vegetative and fruiting tissues of tomato. – J. exp. Bot. **49**: 1173-1181, 1998.

Hew, C.S., Lee, G.L., Wong, S.C.: Occurrence of non-functional stomata in the flowers of tropical orchids. – Ann. Bot. **46**: 195-201, 1980.

Keijzer, C.J., Willemse, M.T.M.: Tissue interactions in the developing locule of *Gasteria verrucosa* during microsporogenesis. – Acta bot. neerl. **37**: 475-492, 1988.

and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. – J. Plant Physiol. **144**: 307-313, 1994.

Khoo, G.H., He, J., Hew, C.S.: Photosynthetic utilization of radiant energy by CAM *Dendrobium* flowers. – Photosynthetica **34**: 367-376, 1997.

Kirichenko, E.B., Chernyad´ev, I.I., Voronkova, T.V., Sokolova, R.S., Doman, N.G.: [Activity of the photosynthesis apparatus in orchids during flowering.] – Fiziol. Rast. **36**: 710-716, 1989. [In Russ.]

Kirichenko, E.B., Krendeleva, T.E., Kukarskikh, G.P., Nizovskaia, N.V.: [Photochemical activity in chloroplasts of anthers and caryopsis pericarp in cereals.] – Fiziol. Rast. **40**: 250-254, 1993. [In Russ.]

Larcher, W.: Ökophysiologie der Pflanzen. 6th Ed. – Ulmer, Stuttgart 2001.

Luthra, Y.P., Sheoran, I.S., Singh, R.: Photosynthetic rates and enzyme activities of leaves, developing seeds and pod-wall of pigeon pea (*Cajanus cajan* L.). – Photosynthetica **17**: 210-215, 1983.

Marcelis, L.F.M., Hofman-Eijer, L.R.B.: The contribution of fruit photosynthesis to the carbon requirement of carbon requirement of cucumber fruits as affected by irradiance, temperature and ontogeny. – Physiol. Plant. **93**: 476-483, 1995.

Mathew, B.: Hellebores. – Alpine Garden Society, W.S. Cowell, Ipswich 1989.

Moreshet, S., Green, G.C.: Photosynthesis and diffusion conductance of the Valencia orange fruit under field conditions. – J. exp. Bot. **31**: 15-27, 1980.

Obeso, J.R.: The costs of reproduction in plants. – New Phytol. **155**: 321-348, 2002.

Salopek-Sondi, B., Kovac, M., Ljubesic, N., Magnus, V.: Fruit initiation in *Helleborus niger* L. triggers chloroplast formation and photosynthesis in the perianth. – J. Plant Physiol. **157**: 357-364, 2000.

Salopek-Sondi, B., Kovac, M., Ljubesic, N., Magnus, V.: Developing fruit direct post-floral morphogenesis in *Helleborus niger* L. – J. exp. Bot. **53**: 1949-1957, 2002.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis. – In: Schulze, E.-D.; Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 49-70. Springer-Verlag, Berlin – Heidelberg – New York 1994.

Smillie, R.M.: Calvin cycle activity in fruit and the effect of heat stress. – Sci. Hortic. **51**: 83-95, 1992.

Vainstein, A., Sharon, R.: Biogenesis of petunia and carnation corolla chloroplasts: changes in the abundance of nuclear and plastid-encoded photosynthesis-specific gene products during flower development. – Physiol. Plant. **89**: 192-198, 1993.

Vemmos, S.N., Goldwin, G.K.: Stomatal and chlorophyll distribution of Cox´s Orange Pippin apple flowers relative to other cluster parts. – Ann. Bot. **71**: 245-250, 1993.

Vemmos, S.N., Goldwin, G.K.: The photosynthetic activity of Cox´s orange pippin apple flowers in relation to fruit setting. – Ann. Bot. **73**: 385-391, 1994.

Watson, M.A., Caspar, B.B.: Morphogenetic constraints on patterns of carbon distribution in plants. – Annu. Rev. Ecol. Syst. **15**: 233-258, 1984.

Weiss, D., Schönfeld, M., Halevy, A.H.: Photosynthetic activities in the *Petunia* corolla. – Plant Physiol. **87**: 666-670, 1988.

Wellburn, A.R.: The spectral determination of chlorophylls *a*

Werner, K., Ebel, F.: Zur Lebensgeschichte der Gattung *Helleborus* L. (Ranunculaceae). – Flora **189**: 97-130, 1994.

Werk, K.S., Ehleringer, J.R.: Photosynthesis by flowers in

Encelia farinosa and *Encelia californica* (Asteraceae). – Oecologia **57**: 311-315, 1983.
Williams, K., Koch, G.W., Mooney, H.A.: The carbon balance of flowers of *Diplacus aurantiacus* (Scrophulariaceae). – Oecologia **66**: 530-535, 1985.