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Seasonal photosynthetic changes in the green-stemmed Mediterranean shrub *Calicotome villosa*: a comparison with leaves

C. YIOTIS[*], G.K. PSARAS, and Y. MANETAS

Section of Plant Biology, Department of Biology, University of Patras, GR 265 00, Patras, Greece

Abstract

Some photosynthetic attributes of leaves and stems were seasonally followed in the small-leaved, summer-deciduous, green-stemmed Mediterranean shrub *Calicotome villosa*. Both leaves and stems displayed similar photon energy-saturated photosystem 2 (PS2) efficiencies with a minimum during winter. A second minimum in stems during the leafless summer period could be ascribed to sustained photoinhibition. Yet, stems were slightly inferior in photon capture, resulting partly from lower chlorophyll (Chl) contents and partly from higher reflectance due to pubescence. As a result, photon energy-saturated linear electron transport rates were slightly higher in leaves. However, when the total leaf and stem areas were taken into account, this superiority was abolished during autumn and winter and more than overturned during spring. Given that during summer the stems were the only photosynthetic organs, the yearly photosynthetic contribution of stems was much higher. Chl contents in stems displayed a transient and considerable summer drop, accompanied by an increase in the carotenoid to Chl ratio, indicating a photo-protective adaptation to summer drought through a decrease of photo-selective capacity, typical for leaves of many Mediterranean plants.

Additional key words: carotenoids; chlorophyll; drought stress; electron transport rates; photoinhibition; photosystem 2 efficiency; spiny broom.

Introduction

Non-foliar photosynthesis may contribute a substantial amount to the plant's net carbon gain under some circumstances. Green parts of flowers (Weiss *et al.* 1988, Aschan and Pfanz 2006, He and Teo 2007), fruits (Blanke and Lenz 1989), stems (Nilsen 1995), or even roots (Benzing *et al.* 1983, Hew *et al.* 1984, Kitaya *et al.* 2002) may be photosynthetically active. We may distinguish between (*a*) green stems and green parts of flowers possessing stomata and displaying high photosynthetic rates assimilating atmospheric CO_2 and (*b*) chloroplast containing tissues hidden within mainly stomata-less organs like secondary stems, most fruits, and fertile flower parts, which accomplish an internal re-fixation of respiratory CO_2 (Aschan and Pfanz 2003).

Green photosynthetic stems represent a common characteristic among non-succulent, xerophytic perennials growing in arid, semi arid, or periodically dry habitats like the Mediterranean-climate areas (Gibson 1983, Comstock and Ehleringer 1990, Nilsen *et al.* 1993). Many of these species are classified as drought deciduous shrubs as their leaves are shed during the dry period. Moreover, the winter leaves are usually small and rather vestigial. Thus, stem photosynthesis may be a substantial source of carbon assimilation during the leafy period of the year and certainly the only source during the dry period (Adams and Strain 1968, Szarek and Woodhouse 1978, Comstock *et al.* 1988, Nilsen *et al.* 1993). The chlorenchymatous cells of green stems are similar to the palisade and/or spongy cells of leaves (Gibson 1983, Comstock and Ehleringer 1988, Yiotis *et al.* 2006) and use the C_3 photosynthetic pathway (Nilsen 1995, Aschan and Pfanz 2003) to assimilate atmospheric CO_2 taken up through the abundant, sunken stomata of the stem epidermis (Gibson 1983, Osmond *et al.* 1987, Yiotis *et al.* 2006). Accordingly, stem and foliar photosynthesis appear to be supported by the same anatomical motif.

So far, studies concerning photosynthesis in green stems have used gas exchange methods to investigate the relative contribution of stems to the net carbon gain of the whole plant (Szarek and Woodhouse 1978, Comstock *et al.* 1988) and the effects of cladode age, nitrogen content, temperature, and water vapour pressure deficit (Osmond *et al.* 1987, Comstock *et al.* 1988, Nilsen *et al.* 1989, Nilsen and Sharifi 1994, Haase *et al.* 1999).

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[*] Author for correspondence; fax: +30 2610 997411, e-mail: chyiotis@upatras.gr

Although green-stemmed, summer-deciduous plants abound in areas of high climate seasonality, the influence of season on stem photosynthesis has not been studied up to now. Corresponding studies with leaves have revealed interesting seasonal acclimation patterns for photosynthesis and photo-protection in several plant species (Beyschlag *et al.* 1987, Kyparissis *et al.* 1997, Munné-Bosch and Alegre 2000). Accordingly, the first question asked in this investigation was whether green-stem photosynthesis under field conditions is equally sensitive to cool winters and particularly to the long, dry, and hot Mediterranean summers. We used as a test plant the green-stemmed, thorny shrub *Calicotome villosa*, which maintains small leaves from November to June (Yiotis *et al.* 2006). Selected photosynthetic attributes were seasonally assessed by chlorophyll (Chl) fluorescence measurement in dark- and light-adapted samples of both leaves and stems. This comparative approach allowed also a crude estimation of the relative contribution of leaves and stems in the total photosynthetic electron flow budget.

Materials and methods

Plants: *Calicotome villosa* (Poiret) Link. (Leguminosae, common name: spiny broom) is a Mediterranean leguminous drought deciduous shrub possessing green, intricately branched stems which terminate in thorny tips. Leaves appear during November after the autumn rains and keep on the plant up to next June. The plants used in this study belong to a population growing in an open field near the campus of Patras University (38°15′N, 21°44′E, 150 m a.s.l.) in north-western Peloponnesus, Greece. Three individuals were tagged and used throughout the study. The measurements lasted from April 2003 to July 2004 with six sampling dates, roughly at the centre of each season.

Chl fluorescence: The photosystem 2 (PS2) photochemical efficiency in the light adapted state [$\Delta F/F_m' = (F_m' - F)/F_m'$ according to Genty *et al.* 1989] was measured with a pulse-amplitude-modulated fluorimeter (*MINI-PAM*, *Walz*, Effeltrich, Germany). Preliminary trials showed that PS2 efficiency was practically similar along the stems and up to 20 cm from the top. The measurements were carried on the exposed, south-facing sides of three stems and three leaves per tagged individual, during mid-day of cloudless days. The dependence of effective PS2 yield on incident photon fluence rates was assessed by triggering the built-in function of the so-called 'rapid light curves'. The program allows the recording of fluorescence responses to steps of increasing and predetermined actinic irradiances of 10-s duration, separated by 0.8-s saturation pulses of 10 000 μmol m^{-2} s^{-1}. “Actinic light” was generated by the internal light source of the instrument. A prerequisite for using this system with light adapted photosynthetic tissues in the field is to shield ambient radiation during measurements. Thus, a dark clip was used. Preliminary trials indicated that lamb output was stable provided that battery voltage was kept between 12.0 and 12.3 V, which is at the plateau region of the capacity *vs.* voltage curve (Handbook of operation, Photosynthesis yield analyzer *MINI-PAM*, 1999). Since the irradiance actually falling on a cylindrical stem cannot be accurately determined, we allowed photon exchange between the instrument and the stems through a 2×10 mm rectangular window fitted on the clip and oriented along the stem axis during the measurements. As the absolute fluorescence signal is reduced in this case, the electronic signal gain of the instrument was changed in order to bring the signal within the range proposed by the manufacturers, while the noise to signal ratio was re-determined.

Yield measurements were used to calculate the linear electron transport rate (ETR) along PS2 according to the formula $\mathrm{ETR} = \Delta F/F_m' \times \mathrm{PAR} \times A \times 0.5$ (Genty *et al.* 1989), where PAR is the incident photosynthetically active irradiance, A is the fraction of incident photons absorbed by the photosynthetic pigments (*i.e.* absorptance), and 0.5 holds for an equal distribution of absorbed photons between the two photosystems. Therefore, the ETR *vs.* PAR curves can be constructed. Rapid irradiance curves are of considerable advantage in quickly assessing the cardinal points of an ETR *vs.* PAR curve (White and Critchley 1999, Rascher *et al.* 2000). In addition, the plots obtained with this technique have a good correspondence with steady-state assessment of ETR at various irradiances, provided that the unavoidable darkening of the tissue before the “actinic light” activation is short enough (*ca.* 30 s, Rascher *et al.* 2000). In our case, tissue darkening was about 5 s. The measured stems were cut at their base (roughly 20 cm from the tip) on the same afternoon, put in air-tight plastic bags, and kept in the dark all night at room temperature. Dark-adapted PS2 photochemical efficiency was measured during next morning as $F_v/F_m = (F_m - F_0)/F_m$. Subsequently, total leaf area (*Li-300A*, *Li-Cor*, USA) and total stem surface area (assessed geometrically through length and radius measurements) for each stem (module) were calculated. The product of area times maximum attained ETR represents the total electron transport capacity of each organ and used for a rough calculation of its relative photosynthetic contribution at each season. Rapid irradiance curves were terminated at roughly 1 600 μmol m^{-2} s^{-1} PAR, as this was the maximum photon fluence rate at leaf or stem surface at mid-day of clear days.

Photosynthetic pigment extraction and quantification: At each sampling date, a number of modules consisting of a 20-cm stem with its leaves were cut and taken immediately to the laboratory for analysis. After measuring leaf and stem surface area as described previously, the stem cortices were stripped off. Chls from leaves and cortex were extracted with mortar and pestle in 100 % methanol plus a pinch of $CaCO_3$ and purified sea sand. After centrifuging the resultant suspensions, Chl *a*, Chl *b*, and total carotenoids (Cars) were determined using a *Shimadzu UV-160-A* double beam spectrophotometer and the equations of Lichtenthaler and Wellburn (1983).

Optical properties: Spectral reflectance (R) of both leaves and stems was measured with a portable spectrometer (*Unispec*, *PP Systems*, Haverhill, MA, USA), equipped with a bifurcated fibre optic cable. The fibre optic was mounted on a black leaf clip in which the plant material was inserted. A *Spectralon* (R>0.97) standard was used for reference. Irradiation was provided through the fibre from the internal halogen source of the spectrometer. Transmittance of PAR was measured by inserting leaves or stripped-off cortex segments between the quantum sensor of the leaf clip of the mini-PAM and an external halogen source. Total visible transmittance (T) was computed from PAR readings in the presence of the stem strips or leaves divided by corresponding readings in their absence. Absorptance (A) was calculated from the formula $A = 1 - R - T$ and used for the calculation of apparent linear electron transport rates.

Statistics: When needed, significance of differences in the measured parameters between leaves and stems was assessed by Student's *t*-test (*SPSS*, Chicago, IL, USA).

Results

Leaf optical properties: Leaf and stem values of R were constant during the leafy period of the year but increased in the stems during the leafless (summer) period. Fig. 1 shows spectral R from leaves and stems obtained during April 2003 and the corresponding curve for stems at mid-summer, while the integrated R, T, and A over the visible spectrum (400–700 nm) are shown in Table 1. Although the spectral R profile of the two organs was similar during April, the absolute values for stems were more than two-fold higher. At mid-summer, stem R was further increased, and the increase was more pronounced in the orange-red band of the spectrum. As a result, the capacity for photon capture (A) of the leaves was roughly 20 % higher than that of stems during the leafy period while stem A was further decreased by *ca.* 13 % during summer.

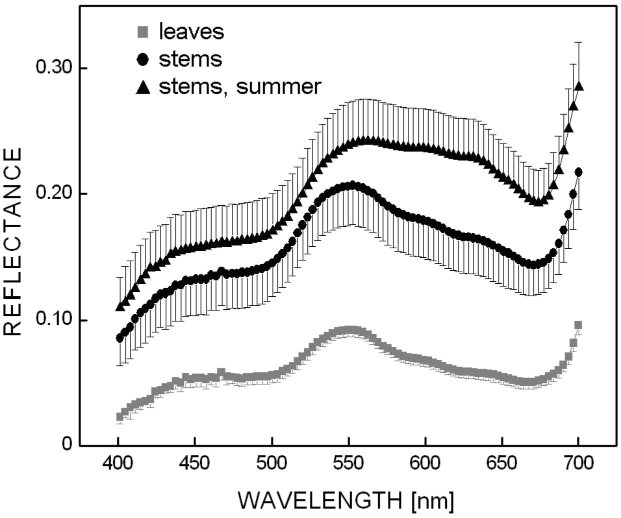

Fig. 1. Spectral reflectance of leaves and stems during the leafy period of the year and that of the stems during the leafless period. Means±SD from 6 independent measurements.

Photosynthetic pigments: Leaves displayed higher area-based Chl and Car contents and a slight trend for higher Chl *a*/*b* ratios, with little and mostly non-significant seasonal changes (Table 2). The only considerable change was a drop in both Chl and Car contents in stems during summer. However, Chl loss was more pronounced than Car loss. Concomitantly, the Car/Chl ratio was considerably increased in stems during summer. When pigment contents were expressed on a branch basis, a different picture emerged. Depending on season, the relative photosynthetic area of each branch allocated to

Table 1. Summary of leaf and stem optical properties (A – absorptance, R – reflectance, T – transmittance) in the visible (400–700 nm) band. "Stem" in this case denotes the stripped-off external part of stems constituted of the chlorophyll-containing cortex plus the pubescent epidermis. Means±SD from 6 independent measurements. Differences in the various parameters between leaves and stems for each sampling date are significantly different ($p<0.05$). The calculated values of A were used for the estimation of ETR.

		April 2003	July 2003	November 2003
R [%]	Leaves	0.062±0.005	–	0.061±0.007
	Stem	0.159±0.027	0.202±0.028	0.178 ± 0.030
T [%]	Leaves	0.007±0.001	–	0.026±0.005
	Stem	0.069±0.009	0.126±0.021	0.081±0.009
A [%]	Leaves	0.93	–	0.91
	Stem	0.77	0.67	0.74

leaves ranged from 17 to 33 % with the lowest values observed in the spring (Table 2). Apparently, branch elongation during spring dilutes the contribution of leaves to the total, effective photosynthetic area. Recalculating the contribution of stem Chls to the total Chl pool of branches, we conclude (Table 2) that stem Chl accounts for 53–67 % of the total during the leafy period and climbs to 100 % during the leafless one. Similar values were obtained for Cars.

Table 2. Area-based photosynthetic pigment contents, pigment ratios, branch-based relative areas of leaves and stems, and contribution of leaves and stem pigments to the total branch pigment content during the course of a year. Chl – chlorophyll, Car – carotenoids. Means±SD from 3 independent measurements. *Different letters for each parameter and each sampling date* indicate significant differences (p<0.05) between leaves and stems.

Variable	Organ	Sampling period			
		November	February	May	July
Chl a+b [mg m^{-2}]	Leaves	826±165^{a}	876±36^{a}	996±156^{a}	–
	Stem	503±64^{b}	609±50^{b}	482±39^{b}	275±35
Cars [mg m^{-2}]	Leaves	119±13^{a}	100±2^{a}	122±12^{a}	–
	Stem	69±7^{b}	83±8^{b}	71±2^{b}	52±6
Chl a/b	Leaves	2.93±0.17^{a}	2.58±0.12^{a}	2.58±0.06^{a}	–
	Stem	2.57±0.13^{b}	2.40±0.05^{a}	2.38±0.05^{b}	2.21±0.02
Car/Chl	Leaves	0.146±0.014^{a}	0.114±0.007^{a}	0.124±0.007^{a}	–
	Stem	0.138±0.005^{a}	0.135±0.003^{b}	0.148±0.012^{b}	0.191±0.034
% relative surface area	Leaves	31.0±4.9^{a}	32.5±5.3^{a}	17.0±6.4^{a}	–
	Stem	69.0±4.9^{b}	67.5±5.3^{b}	83.0±6.4^{b}	100.0
% relative Chl content	Leaves	46.7±4.1^{a}	45.4±6.7^{a}	33.4±12.0^{a}	–
	Stem	53.3±4.1^{a}	54.6±6.7^{a}	66.6±12.0^{b}	100.0
% relative Car content	Leaves	48.1±5.8^{a}	41.3±7.3^{a}	29.5±10.0^{a}	–
	Stem	51.9±5.8^{a}	58.7±7.3^{b}	70.5±10.0^{b}	100.0

PS2 photochemical efficiency: Photon-saturated PS2 photochemical efficiency followed a bi-modal pattern with peaks during autumn and spring and troughs at winter (leaves and stems) and summer (stems) (Fig. 2*A*). Note that leaves are absent in summer. In no case a significant difference between leaves and stems was observed. Calculated maximum linear electron transport rates (ETR) followed the same seasonal pattern in both organs. Yet, in spite of the similarity in PS2 effective yield, leaves maintained a slightly, yet significantly, higher area based ETR during April 2003 and January 2004 (Fig. 2*B*) as a result of their higher absorptive capacity (Table 1). Maximum (dark-adapted) PS2 efficiency remained optimal (0.79–0.85) throughout the leafy period for both organs, but displayed a considerable drop to 0.73 for stems at mid-summer (Fig. 2*C*).

Fig. 3 shows the relative contribution of each organ to the total branch ETR activity. For this estimation, the use of projected leaf area and total cortex area (as in the case of photosynthetic pigments) was not justified, since the photosynthetic contribution of the lower leaf surface (triggered by diffuse radiation) is not taken into account. On the other hand, the corresponding contribution of the inner surface of cortex may not be appreciable, since diffuse radiation in the shaded stem side is filtered by overlying cell layers. Hence, for the relative contribution of each organ to the total branch ETR, the leaf projected area was doubled. As shown in Fig. 3, stems and leaves almost equally contributed during autumn and winter while stems were superior in spring. Apparently, the contribution of stems to the whole plant ETR jumped to 100 % during the leafless summer period.

Discussion

Both leaves and stems of *C. villosa* display a bi-modal profile of seasonal photosynthetic linear electron transport rates, with peaks and troughs at the same periods. It seems also that shedding leaves in the summer is not enough for drought avoidance, since evergreen stems display a second photosynthetic minimum during this period (Fig. 2). The bi-modal pattern of photosynthesis in evergreen Mediterranean plants has been repeatedly reported (Beyschlag *et al.* 1987, Grammatikopoulos *et al.* 1995, Kyparissis *et al.* 1997, Martinez-Ferri *et al.* 2000, 2004, Ain-Lhout *et al.* 2004), yet the extent of PS2 efficiency loss as well as the relative contribution of dynamic or sustained photoinhibition is both species- and season-specific (Karavatas and Manetas 1999, Martinez-Ferri *et al.* 2000, 2004). In the case of our test plant, a dynamic down-regulation of PS2 activity due to low winter temperatures can be inferred for both leaves and stems, yet a partial, more permanent PS2 damage in stems is evident during the summer, when the PS2 maximum efficiency is not fully recovered at night

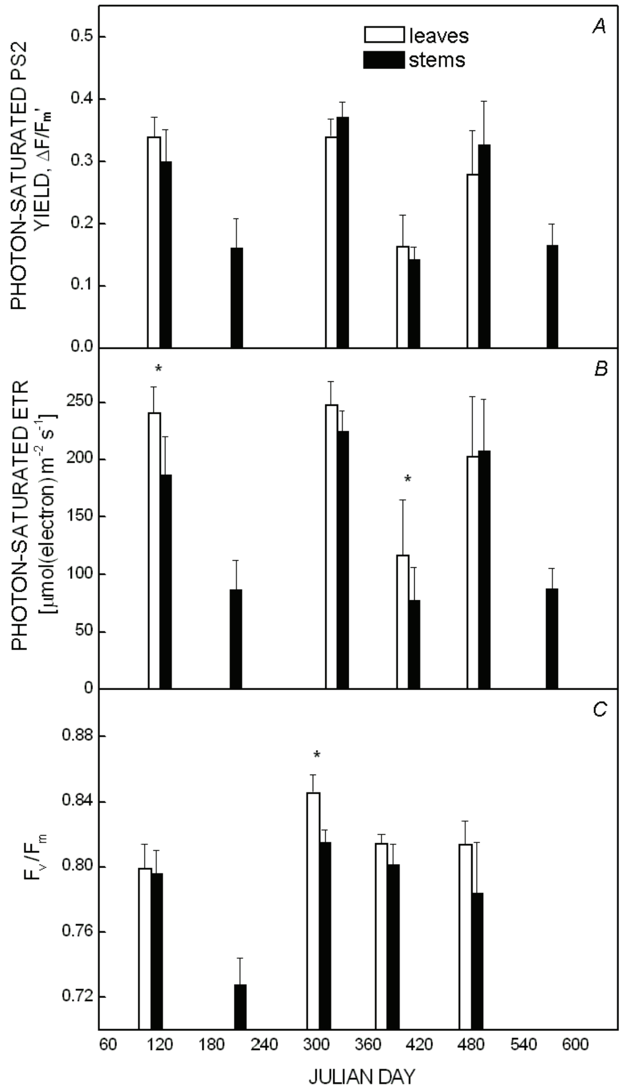

Fig. 2. Seasonal fluctuations in dark-adapted (pre-dawn, *C*) and light-saturated (mid-day, *A*) photosystem 2 (PS2) yield and corresponding maximum linear electron transport rates (*B*) of leaves and stems. Means±SD from 9 independent measurements. *The asterisks* denote significant differences ($p<0.05$) between leaves and stems at the corresponding sampling dates.

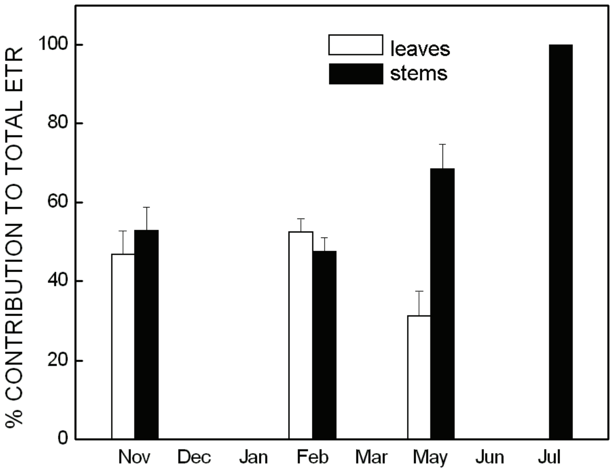

Fig. 3. Seasonal contribution of leaves and stems to the total, branch-based electron transport activity. See text for calculation details.

(Fig. 2). Apparently, summer leaf fall and the xerophytic characteristics of photosynthetic stems (sunken stomata and abundant trichomes, Yiotis *et al.* 2006) do not afford enough protection from drought, allowing chronic photoinhibition to occur. Within this context, we consider that stem Chl loss during the summer may be adaptive, preventing further PS2 damage by reducing excitation load. Note that Car loss is much less pronounced (Table 2), hence the ratio of photo-protective (Cars) to photo-selective (Chls) pigments increases during the summer. Transient summer reduction of light-harvesting capacity but maintenance of photo-protective ability is displayed by leaves of several evergreen Mediterranean plants and assumed to be photo-protective (Kyparissis *et al.* 1995, 2000, Munné-Bosch and Alegre 2000, Balaguer *et al.* 2002).

Our results also showed that during the leafy period of the year, the irradiance-adapted PS2 efficiency of stems equals that of leaves, although maximum ETR is somewhat restricted due to lower photon capture, resulting from lower Chl contents and higher reflectance in the pubescent stems. Yet, the seasonally integrated contribution of stems to total branch photosynthesis is finally higher due to enhanced actual stem photosynthetic areas in the spring and the absence of leaves in the summer.

In conclusion, although green stem photosynthesis in *C. villosa* is sensitive to both winter cold and summer drought, it outcompetes leaf photosynthesis on an annual basis.

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