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Research paper

A novel pattern of leaf movement: the case of *Capparis spinosa* L.

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A novel type of heliotropic leaf movement is presented for *Capparis spinosa* L., a summer perennial shrub of Mediterranean and arid ecosystems. In contrast to plants that demonstrate uniform diaheliotropic and/or paraheliotropic movement for all their foliage, the alternate leaves of *C. spinosa* follow different movement patterns according to their stem azimuth and the side of the stem that they come from (cluster). Additionally, leaf movement for each cluster may not be uniform throughout the day, showing diaheliotropic characteristics during half of the day and paraheliotropic characteristics during the rest of the day. In an attempt to reveal the adaptive significance of this differential movement pattern, the following hypotheses were tested: (i) increase of the intercepted solar radiation and photosynthesis, (ii) avoidance of photoinhibitory conditions, (iii) amelioration of water-use efficiency and (iv) adjustment of the leaf temperature microenvironment. No evidence was found in support of the first two hypotheses. A slight difference toward a better water use was found for the moving compared with immobilized leaves, in combination with a better cooling effect.

Keywords: caper, heliotropic leaf movement, light-use efficiency, photoinhibition, photosynthesis, water-use efficiency.

Introduction

Among the numerous plant movements, ranging from the sub-cellular level to the whole-plant organ level, heliotropic leaf movements have a dominant position and role in light interception and consequently plant growth. Much research has been devoted to the description of leaf movement types (Ehleringer and Forseth 1980, Koller 2000), addition of new species to the list of plants performing movements (Sailaja and Rama Das 1996), study of signal perception and the mechanism of movements (Coté 1995, Koller 2000) and environmental factors driving leaf movements (Kao and Forseth 1991, Bielenberg et al. 2003, Pastenes et al. 2004, 2005, Arena et al. 2008).

Heliotropic leaf movements refer to the ability of leaves of many plant species to track the sun's position in the sky and move in response to its diurnal change. Two main kinds of diurnal movements are recognized: diaheliotropic movements in

which the leaf lamina remains oriented perpendicular to the sun's direct rays and paraheliotropic movements in which the leaf lamina is oriented obliquely or, in extreme cases, parallel to the sun direct rays (Ehleringer and Forseth 1980). Heliotropic movements are characterized by rapidity, reversibility and by overnight resetting to face the morning sun. The first and most important consequence of leaf solar tracking is to regulate the level of the incident photon irradiance from the sun. From an ecophysiological point of view, it is more correct to refer to this regulation as an optimization of interception of photosynthetically active radiation and not necessarily as maximization or minimization of it.

Paraheliotropism has been described as a stress avoidance mechanism: excess light avoidance confers to the leaves the capacity to protect against photoinhibition, by reducing excitation pressure to reaction centers and heat (Kao and Tsai 1998, Pastenes et al. 2005, Jiang et al. 2006, Arena et al. 2008). Recently, it has been proposed that leaves move to remedy the

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deficiency of physiological photoprotection for photosystem II, thus a decrease in PSII activity (i.e., PSII photoinhibition) regulates the degree of leaf movement under environmental stresses (Huang et al. 2012, 2014). Paraheliotropism can also be considered an effective means to reduce leaf temperature and ameliorate plant resistance to drought conditions by limiting transpirational water loss (Gamon and Pearcy 1989, Bielenberg et al. 2003). Finally, paraheliotropic leaf movements allow a compromise between light interception and stress avoidance to maximize carbon gain (Arena et al. 2008, Huang et al. 2014). In an ecological/ecophysiological context, many studies have examined the role of certain environmental factors in modifying heliotropic responses. Especially for paraheliotropism, it is well documented that soil water availability (Kao and Tsai 1998, Pastenes et al. 2004, 2005), air temperature (Fu and Ehleringer 1989), photosynthetic photon flux (Berg and Heuchelin 1990) and nitrogen availability (Kao and Forseth 1991), singly or in combination, may play a role in paraheliotropic movements, determining their occurrence (Rajendrudu et al. 1996) and degree (Kao and Tsai 1998, Bielenberg et al. 2003, Pastenes et al. 2005). Steeper leaf angles as a result of low water and nitrogen availability or high air temperature and photon flux confirm the role of paraheliotropism as a stress-alleviating mechanism. The pronounced relation (combination or even complementation) between water availability and leaf movement is depicted in the case of Townsville stylo (*Stylosanthes humilis* Kunth), a plant with diaheliotropic movements under well-watered conditions and paraheliotropic movements under drought conditions (Begg and Jarvis 1968).

Diaheliotropism, on the other hand, results in relatively constant high photon irradiances, enabling rapid photosynthetic rates throughout the day. Diaheliotropic leaves probably have the physiological ability, especially a high light-saturation point, for a more efficient utilization of high light (Ehleringer and Forseth 1980, Sailaja and Rama Das 1996, Greer and Thorpe 2009). Few studies have focused on how diaheliotropic plants cope with overheating and the risk of photoinhibition, side effects of light harvesting maximization throughout the day (Greer and Thorpe 2009, Zhang et al. 2009, Zhu et al. 2015). It is reported that some diaheliotropic leaves have a strong capacity to dissipate excessive irradiance that may damage photosynthetic apparatus by non-photochemical energy quenching (Zhang et al. 2009, Zhu et al. 2015). Sailaja and Rama Das (1996) have suggested that the capacity for high photosynthetic rates per se functions as a dissipative mechanism at super-optimal irradiances. Presumably, this is the case of high-light demanding species like *Malva parviflora* L., which has proved to be relatively resistant to photoinhibition (Greer and Thorpe 2009).

Capparis spinosa L. is a summer perennial shrub growing in the Mediterranean and semiarid ecosystems (Sozzi 2001). It usually thrives in rocky and anhydrous habitats, fully exposed to the sun's rays and under extremely high temperatures. Its annual

above ground growth commences during the end of spring (May) and concludes during early autumn (October), i.e., during the period of the year with minimum precipitation and maximum temperature. These adverse conditions are further enhanced by high light intensities, which prevail throughout the summer period. The combination of the three above-mentioned environmental parameters may establish photo-oxidative conditions and this is the case for many sclerophyllous and semi-deciduous plants typical of Mediterranean ecosystems (Kyparissis et al. 1995, Karavatas and Manetas 1999, Larcher 2000). Nevertheless, *C. spinosa* does not seem to suffer any summer stress (Levizou et al. 2004), while preliminary observations indicated that its leaves move on a daily basis. The purpose of this study is to demonstrate the leaf movement pattern and furthermore to investigate the possible adaptive role of this movement.

Materials and methods

Capparis spinosa

Capparis spinosa canopy is made of several radial decumbent branches with secondary stems growing from them. Plants expand horizontally, usually do not exceed 50 cm in height and may occupy an area of 15 m². Accordingly, the whole canopy has full access to direct sunlight throughout the day, with very low shading between leaves. All measurements were performed on mature individuals of *C. spinosa* growing wild in the vicinity of the Patras University campus, Greece (38.29098°N, 21.79042°E), during clear sky days.

Movement

Leaves of *C. spinosa* grow in an alternate pattern. Accordingly, leaves belonging to one stem may be grouped into two clusters, corresponding to the side of the stem that they come from. For the present study, the leaves of stems with different azimuth (the angle between the stem and north) were tagged and used for all measurements. Stems with azimuth around 0° and 180° have a north–south orientation, i.e., perpendicular to the ground projected solar trajectory, while stems with azimuth around 90° and 270° have an east–west orientation, i.e., in parallel to the ground projected solar trajectory.

For movement description, leaf inclination and leaf azimuth measurements were performed. Leaf inclination was measured as the angle between the lamina plane and the horizontal using a protractor with a weight hanging from it, having an accuracy of 5° (0°, horizontal leaf; 90°, vertical leaf) (Prichard and Forseth 1988). Leaf azimuth was measured using a map compass with 5° accuracy according to Forseth and Ehleringer (1980). The cosine of incidence ($\cos(i)$) between the leaf plane and a normal to the sun's direct beam was computed according to the formula (Prichard and Forseth 1988):

$$\cos(i) = \cos(\beta)\cos(z) + \sin(\beta)\sin(z)\cos(a_s - a_l),$$

where β is the leaf angle from the horizontal, z is the solar zenith angle, α_s is the solar azimuth angle and α_l is the leaf azimuth angle.

$\cos(i)$ is a measure of the proportion of the direct sun beam incident on a leaf and ranges between -1 and 1 (Lang 1973). Values around 1 correspond to leaves with their adaxial surface perpendicular to the sun's rays, whereas values around 0 correspond to leaves parallel to sun rays. Negative values correspond to leaves with their adaxial surface no longer intercepting direct sunlight.

Movement measurements were performed during 2000 (five dates), 2002 (four dates) and 2005 (six dates), between June and August. Each year four to six individuals were selected; in each individual four stems with different azimuth were selected and on each stem six to eight leaves were tagged, half of them coming from the one side of the stem and half of them from the other (clusters 1 and 2). Measurements were performed at approximately 1:45 h intervals, from pre-dawn to sunset.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured at the same plants/tagged leaves used for movement measurements, during 2002 and 2005. Chlorophyll a fluorescence parameters, leaf temperature and photosynthetic active radiation (PAR) were measured in vivo with a Mini-PAM pulse-amplitude modulated fluorimeter equipped with a 2030-B Leaf-Clip Holder (Walz, Effeltrich, Germany). All measurements were completed within 1–2 s and were performed at the natural orientation of each leaf. The recordings of the instrument's PAR sensor (measuring at leaf level) and leaf temperature sensor were used for describing leaf light and temperature environment, respectively, over the course of the day.

Photochemical efficiency of PSII was calculated according to Genty et al. (1989) as

$$\frac{\Delta F}{F'_m} = \frac{(F'_m - F_s)}{F'_m}$$

Non-photochemical quenching (NPQ) was calculated from the Stern–Vollmer relationship according to Bilger and Björkman (1990) as

$$\text{NPQ} = \frac{F_m}{F'_m} - 1$$

For NPQ calculation of each leaf throughout the day, its pre-dawn F'_m value was used as F_m .

Gas exchange

Gas exchange parameters were measured at the same plants/tagged leaves used for movement and chlorophyll fluorescence measurements, during 2005. Gas-exchange measurements (CO_2 , H_2O) were performed in the field with a portable photosynthesis system (LCpro+, ADC BioScientific Ltd, Hoddesdon, UK)

under natural sunlight and in ambient concentrations of CO_2 and H_2O . The climate control capability of the instrument was used for keeping the leaf chamber temperature in accordance with ambient temperature ± 1 °C. All measurements were made at the natural orientation of each leaf.

Immobilization

In 2005, the leaves of one additional stem per plant with north–south orientation were immobilized and used in parallel with the leaves of the four stems per plant, for all previous measurements. Immobilization was performed during the afternoon before measurement using pins and threads. Leaves of both clusters were stabilized in a horizontal orientation.

Water potential

Shoot water potential (Ψ) was measured at the same plants used for all previous measurements, during 2005. Measurements were performed in the field with a portable Scholander-type pressure chamber (SKPM 1400–80, Skye Instruments Ltd, Llandrindod Wells, UK), with a -8 MPa measuring limit. For each measurement, three randomly selected shoots were wrapped in aluminium foil, sealed in plastic bags and after 10 min were cut and measured immediately. Due to the very short leaf petiole, it was not possible to perform single leaf water potential measurements.

Meteorological data

Meteorological data (air temperature) were recorded by a solar powered Mini-Met (Skye Instruments) meteorological station located in the study area on a 10 min basis.

Statistical analyses

Statistical significance for the parameter differences between stems with different azimuths (Table 1) was examined using the Kruskal–Wallis non-parametric test followed by Dunn's multiple comparisons procedure, with significance level set to $P < 0.05$. The statistical analyses were performed with the statistical package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Movement

Movement measurements were performed in several plants during three different years (2000, 2002 and 2005). The general movement pattern described below was confirmed for all individuals during all years. However, since differences in stem azimuth were inevitable between individuals and years, and since the movement pattern depends strongly on stem azimuth (see below), no statistical analysis between individuals and/or years was attempted. Instead, the results are presented for a single individual and a single date, focusing on the differences between stems and clusters.

Table 1. Daily integrals of PAR, photosynthesis (A), LUE, transpiration (E), WUE and difference between air and leaf temperature (ΔT), for normally moving (stems 1–4) and immobilized leaves. For each stem, data are given per cluster and as a stem average. Data are averages \pm SD from three to four leaves per cluster and six to eight leaves per stem. Differences were examined for stem average values and different letters between columns indicate statistically significant differences at $P < 0.05$ level.

	Stem 1	Stem 2	Stem 3	Stem 4	Immobilized
PAR, mol m ⁻²					
Cluster 1	35.7 \pm 7.0	38.4 \pm 2.5	35.8 \pm 1.7	35.3 \pm 3.6	44.8 \pm 0.9
Cluster 2	54.3 \pm 0.6	50.3 \pm 0.8	55.8 \pm 1.3	54.4 \pm 0.6	46.0 \pm 0.3
Stem average	45.0 \pm 11.1	44.4 \pm 6.7	45.8 \pm 11.0	44.9 \pm 10.7	45.4 \pm 0.9
A , mol m ⁻²					
Cluster 1	0.82 \pm 0.22	1.01 \pm 0.05	0.88 \pm 0.04	0.91 \pm 0.05	1.01 \pm 0.03
Cluster 2	1.25 \pm 0.06	1.05 \pm 0.06	1.32 \pm 0.08	1.26 \pm 0.12	1.09 \pm 0.07
Stem average	1.04 \pm 0.28	1.03 \pm 0.05	1.10 \pm 0.25	1.08 \pm 0.21	1.05 \pm 0.07
LUE, mol CO ₂ , mol ⁻¹ PAR					
Cluster 1	0.023 \pm 0.003	0.026 \pm 0.002	0.025 \pm 0.001	0.026 \pm 0.001	0.023 \pm 0.001
Cluster 2	0.023 \pm 0.001	0.021 \pm 0.002	0.024 \pm 0.001	0.023 \pm 0.002	0.024 \pm 0.001
Stem average	0.023 \pm 0.002	0.024 \pm 0.003	0.024 \pm 0.001	0.024 \pm 0.002	0.023 \pm 0.001
E , mol m ⁻²					
Cluster 1	254.7 \pm 30.5	288.3 \pm 13.1	326.5 \pm 10.7	321.6 \pm 6.3	323.2 \pm 31.1
Cluster 2	346.5 \pm 24.0	332.0 \pm 16.0	374.6 \pm 33.2	355.4 \pm 24.9	405.4 \pm 60.1
Stem average	300.6 \pm 55.9	310.1 \pm 27.3	350.6 \pm 34.4	338.5 \pm 24.6	364.3 \pm 62.1
WUE, mol CO ₂ , mol ⁻¹ H ₂ O					
Cluster 1	0.0032 \pm 0.0005	0.0035 \pm 0.0000	0.0027 \pm 0.0002	0.0028 \pm 0.0002	0.0031 \pm 0.0004
Cluster 2	0.0036 \pm 0.0001	0.0032 \pm 0.0001	0.0035 \pm 0.0002	0.0035 \pm 0.0001	0.0027 \pm 0.0004
Stem average	0.0034 \pm 0.0004	0.0033 \pm 0.0002	0.0031 \pm 0.0005	0.0032 \pm 0.0004	0.0029 \pm 0.0004
ΔT , °C days					
Cluster 1	1.63 \pm 0.18	1.49 \pm 0.16	1.07 \pm 0.01	1.22 \pm 0.41	0.98 \pm 0.23
Cluster 2	1.53 \pm 0.17	1.28 \pm 0.21	0.70 \pm 0.18	1.12 \pm 0.11	0.75 \pm 0.03
Stem average	1.58 \pm 0.17 ^a	1.39 \pm 0.20 ^{ab}	0.89 \pm 0.24 ^b	1.17 \pm 0.28 ^{ab}	0.86 \pm 0.19 ^b

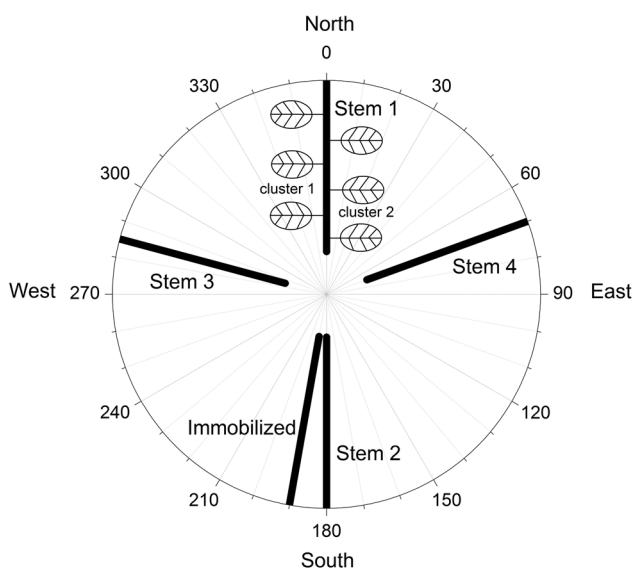


Figure 1. Polar plot of the stems with normally moving (stems 1–4) and immobilized leaves, indicating their azimuth (°). For stem 1, leaves belonging to the two clusters are also shown.

In Figure 1, a graph with the stems used for all measurements in their natural orientation is shown. A typical arrangement of the alternate leaves in two clusters is also shown for stem 1.

Stems 1, 2 and 'Immobilized' had a north–south orientation, while stems 3 and 4 an east–west. Leaves on stems 1–4 were freely moving, while the ones on the 'Immobilized' stem were restricted as described in Materials and methods.

Measurements revealed that leaves are moving, and furthermore that the movement pattern is different between stems with different azimuth and also between the two clusters of each stem (Figure 2). Additionally, leaf movement for each cluster may not be uniform throughout the day.

Stems with north–south orientation As shown in Figure 2, for stems with a north–south orientation (stems 1 and 2), leaf movement concerns mainly lamina inclination (Figure 2a and b), while azimuth angle remains almost stable throughout the day (Figure 2f and g). For these stems, leaves belonging to cluster 1 are almost vertical in the morning and move towards a horizontal orientation in midday and the afternoon (Figure 2a and b; Figure 3). As judged by the diurnal fluctuation of $\cos(i)$ for these clusters (cluster 1 of stems 1 and 2, Figure 2k and l), leaf movement presents diheliotropic characteristics in the morning, changing to paraheliotropic in midday–early afternoon. As a result of this movement pattern, leaves of these clusters are accepting higher light intensities than they would being horizontal early in the morning and lower in the afternoon (Figure 2p

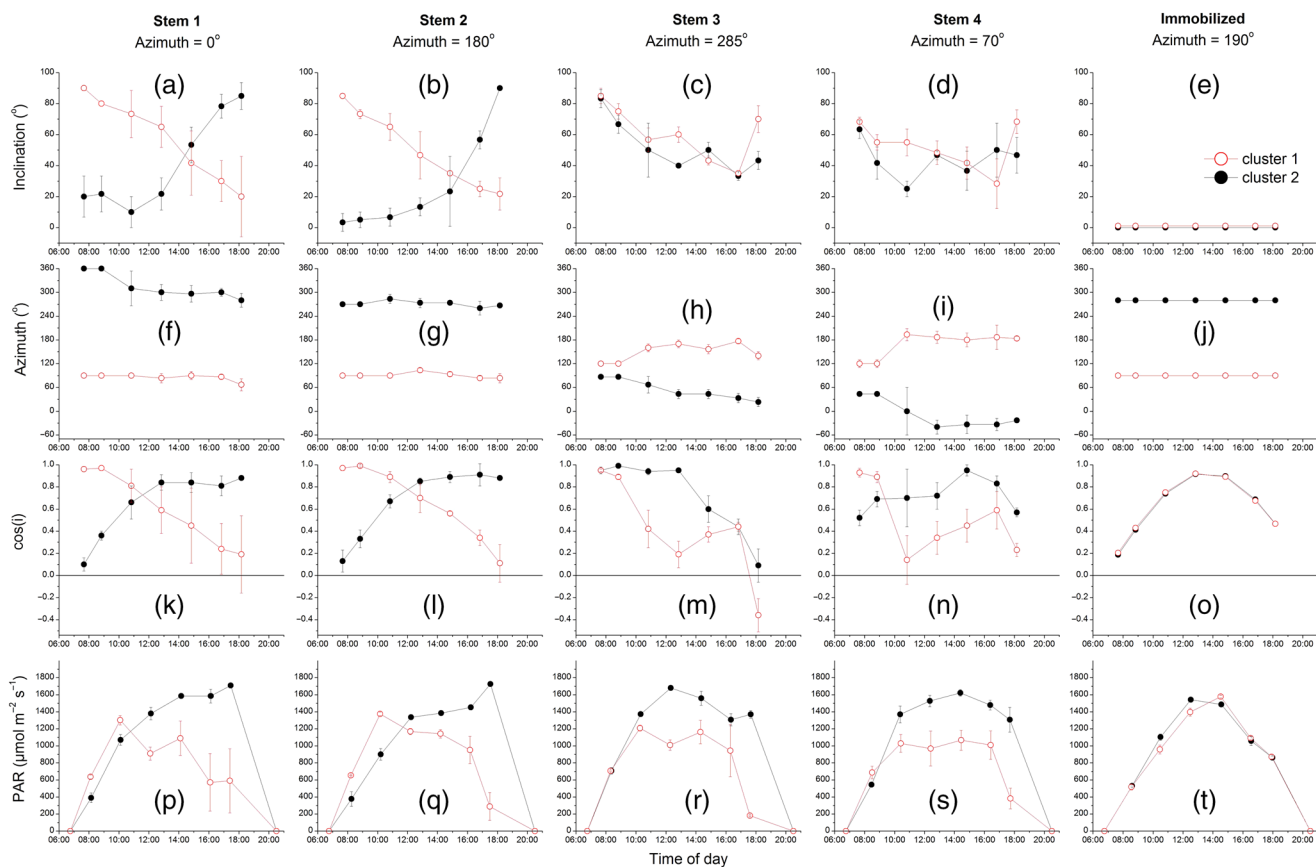


Figure 2. Daily fluctuation of leaf inclination, leaf azimuth, $\cos(i)$ and photosynthetic active radiation (PAR) for normally moving (stems 1–4) and immobilized leaves. Error bars denote SD.

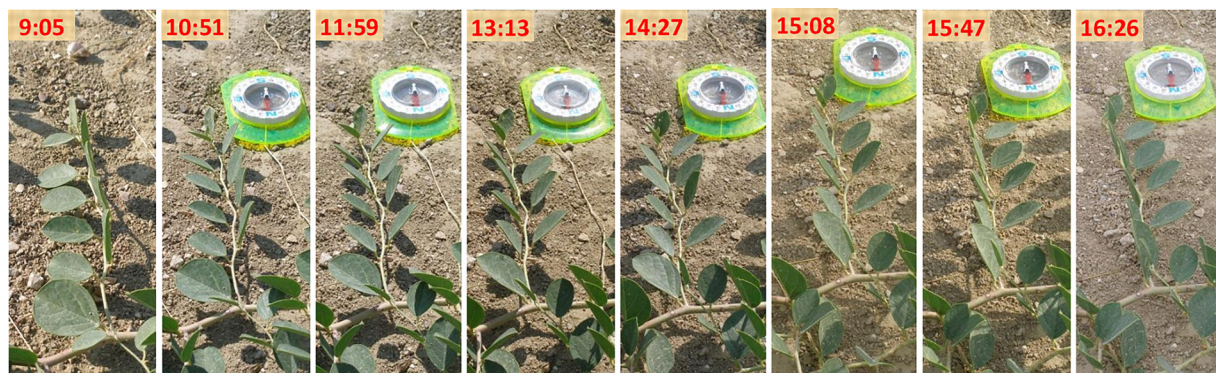


Figure 3. Photographs of a *C. spinosa* stem with north–south orientation during the course of the day. The leaves of the two clusters can be distinguished: leaves of cluster 1 are the vertical ones at the 9:05 photograph and leaves of cluster 2 the horizontal ones.

and q, compare with immobilized leaves Figure 2t). Leaves of cluster 2 on the same stems follow a reverse movement pattern. They are almost horizontal in the morning, turning to vertical in the midday and afternoon (Figure 2a and b; Figure 3). Accordingly, their $\cos(i)$ fluctuation indicates paraheliotropic movement characteristics in the morning hours, turning to diaheliotropic during midday and the afternoon (Figure 2k and l). As a result, the irradiance intercepted by these leaves is lower than that

the horizontal level in the morning and higher in the afternoon (Figure 2p and q, compare with immobilized leaves Figure 2t).

Stems with east–west orientation A different movement pattern is followed by leaves belonging to stems with east–west orientation (Figure 2, stems 3 and 4). Compared with leaves on stems with a north–south orientation (stems 1 and 2 described above), leaves on stems 3 and 4 show lower change in their

lamina inclination (Figure 2c and d) and higher change in their azimuth (Figure 2h and i). Even though their daily $\cos(i)$ fluctuation does not present such a clear pattern as in stems 1 and 2, strong fluctuations still exist, with both paraheliotropic and diaheliotropic characteristics during the course of the day (Figure 2m and n). As a result, all leaves of the one cluster accept irradiance higher than that at the horizontal level, while leaves of the other cluster accept irradiance lower than that at the horizontal level throughout the day (Figure 2r and s, compare with immobilized leaves Figure 2t).

Adaptive significance

In an attempt to reveal the adaptive significance of the peculiar movement pattern described above, several hypotheses were examined.

Daily intercepted PAR and photosynthesis Among the roles ascribed to diaheliotropism, the increase of the intercepted PAR and thus photosynthetic productivity on a daily basis are of major importance. To that purpose, PAR (Figure 2p–t) and photosynthesis (Figure 4a–e) measurements were performed throughout the day on normally moving and immobilized leaves, while light-use efficiency (LUE) was calculated as the ratio of photosynthe-

sis to PAR (Figure 4f–j). Accordingly, the daily integrals of the three parameters were calculated per cluster and stem (Table 1), setting zero values at sunrise and sunset. No statistically significant differences appear between normally moving and immobilized leaves on any of the three parameters (Table 1).

Risk of photoinhibition Another role—mainly related to paraheliotropism—is the avoidance of high light intensities, which may lead to photoinhibitory conditions. However, photosynthesis values as high as $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 4a–e) at air temperatures over 40°C (Figure 5k–o) cannot be considered indicative of photoinhibitory problems. Additionally, even though the movement of leaves leads to a time shift of maximum PAR (Figure 2p–t) and photosynthesis (Figure 4a–e) early in the morning or late in the afternoon (at least for stems with a north–south orientation) compared with that of immobilized leaves, relatively high values of both parameters are still apparent for many clusters during midday. Nevertheless, to further test this hypothesis, chlorophyll fluorescence was measured on a daily basis for moving and immobilized leaves, to evaluate the status of the photosynthetic apparatus from both the photochemical (judged from F_v/F_m , Figure 5a–e) and the non-photochemical (judged from NPQ, Figure 5f–j) point of view. It was found that leaf movement

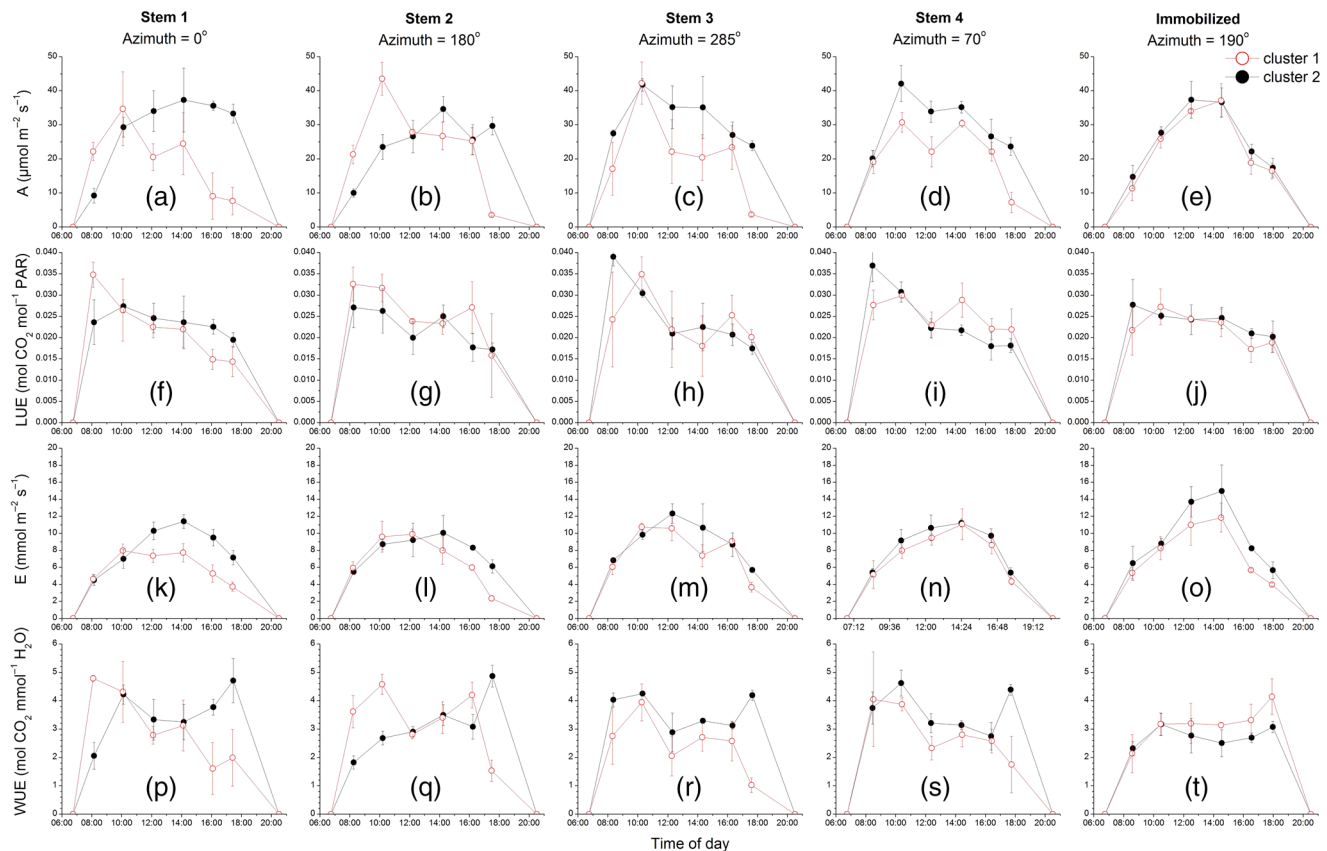


Figure 4. Daily fluctuation of photosynthesis (A), LUE, transpiration (E) and WUE for normally moving (stems 1–4) and immobilized leaves. Error bars denote SD.

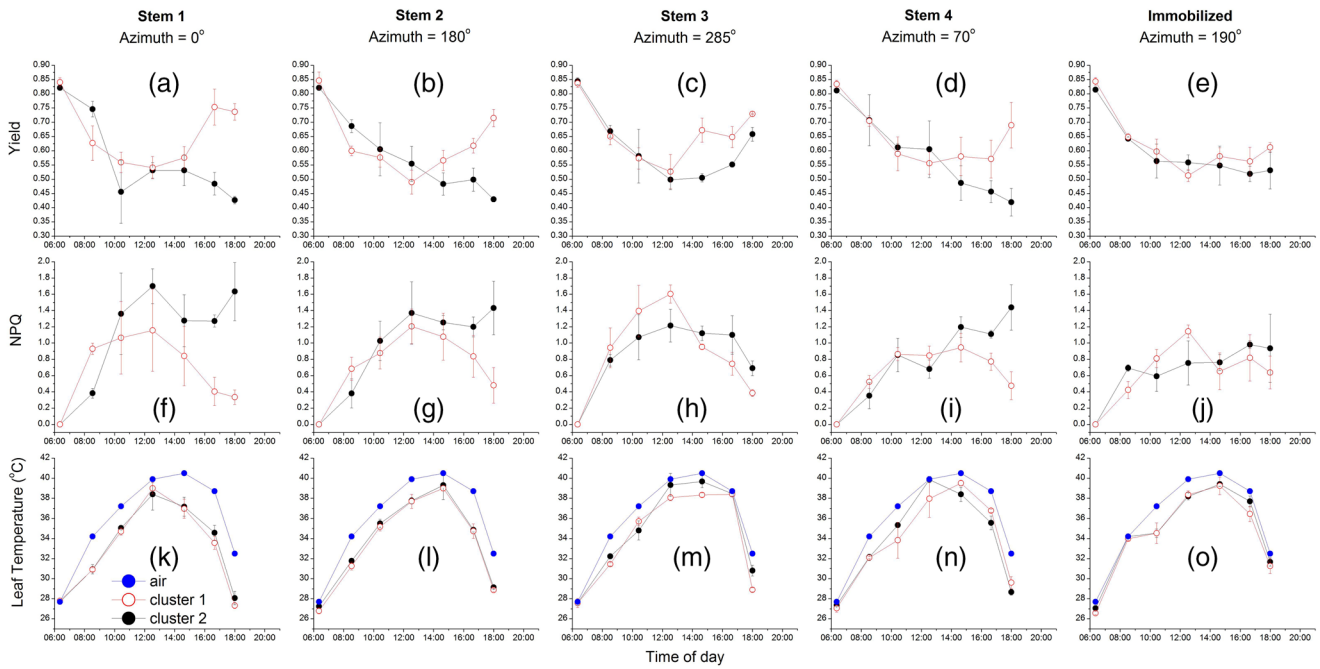


Figure 5. Daily fluctuation of PSII photochemical efficiency (Yield), NPQ and leaf and air temperature for normally moving (stems 1–4) and immobilized leaves. Error bars denote SD.

did not offer any beneficial effects either on photochemical or on non-photochemical quenching. On the contrary, especially the NPQ values appear higher on the moving leaves compared with that of the immobilized ones.

Water-use economy Another hypothesis for the adaptive significance of moving leaves may be related to water management. To test this hypothesis, transpiration rates were measured on a daily basis and water-use efficiency (WUE) was calculated as the photosynthesis to transpiration ratio. As shown in Figure 4k–o, moving leaves—especially the ones on stems with north–south orientation—tend to transpire less water over the course of the day compared with immobilized ones, resulting in higher WUE values (Figure 4p–t), but none of these differences is statistically significant on a daily basis (Table 1). Even if that was the case, the consideration of the movement pattern as a response toward better water economy is not justified by water status measurements (Figure 6). Even though it was not possible to measure water potential for the leaves of the different clusters, the general water status of the plant is not indicative of a stress situation. Indeed, pre-dawn water potential was measured around -1 MPa, falling at -2.3 MPa during midday, which is a rather high value for the Mediterranean environment during the mid-summer period. Additionally, water potential almost fully recovered during late afternoon, confirming adequate access to deep water resources.

Temperature An additional role related mainly to paraheliotropism is the amelioration of the temperature microenvironment at

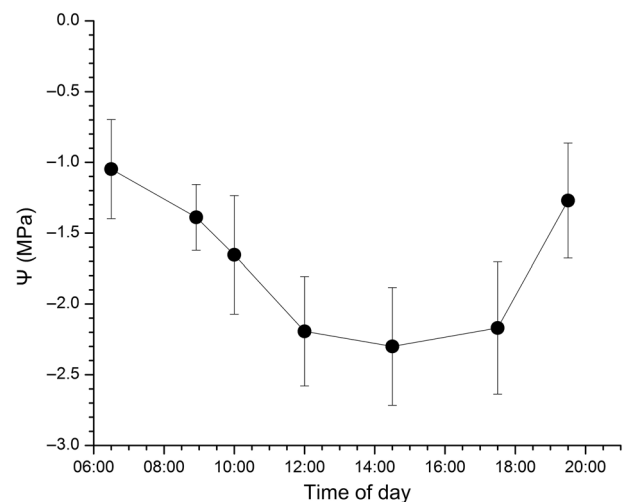


Figure 6. Daily fluctuation of shoot water potential (Ψ). Error bars denote SD.

leaf level. It seems that caper's well-documented unrestricted access to water supplies supporting high transpiration rates as shown above results in effective transpirational cooling (Figure 5k–o) that may reach up to 3.5 °C during extremely hot days (Levizou et al. 2004). As shown in Figure 5, all moving and immobilized leaves maintain lower than air temperature throughout the day. However, leaves in stems with north–south orientation (stems 1 and 2), which present the most intense and clear moving pattern, seem to attain a better cooling effect despite the fact that they transpire less water on a daily basis (Table 1). This may be explained by the movement pattern in these stems,

which results in maximum intercepted PAR early in the morning (Figure 2p and q, cluster 1) or in the afternoon (Figure 2p and q, cluster 2) and not during midday as in the immobilized leaves (Figure 2t).

Discussion

In the present study, a novel non-uniform (differential) diurnal leaf movement pattern is presented for the summergreen *C. spinosa*. Leaf movement is differentiated according to the stem azimuth and the side of the stem that leaves come from (cluster). Additionally, leaf movement for each cluster is not uniform throughout the day, but may present diaheliotropic characteristics during part of the day and paraheliotropic characteristics during the rest of the day. Leaves on stems with a north–south orientation mainly alter their inclination, while the ones on stems with an east–west orientation alter both inclination and azimuth.

Heliotropic leaf movements have been described for several species during the last two centuries (Darwin 1880, Ehleringer and Forseth 1980). Studies conducted so far concern movements that are followed by all the leaves of one plant (uniform type of movement), either tracking (diaheliotropism) or avoiding (paraheliotropism) the sun. Additionally, a heliotropic plant may change its movement pattern from the one type to the other as a response to a gradually imposed stress (i.e., water shortage, Begg and Jarvis 1968) or on a diurnal basis (forenoon and afternoon, Zhu et al. 2015). Moreover, differences in leaf inclination between young and mature soybean leaves, as well as during different plant growth stages, have been reported (Ikeda and Matsuda 2002, Jiang et al. 2006). Even in that case, the movement pattern remains uniform for all leaves. To the best of our knowledge, the only exception to this uniform pattern is *Styrax camporum* Pohl., a native shrub from the Brazilian savanna: most of its leaves are diaheliotropic, whereas some are paraheliotropic, mainly at noon (Habermann et al. 2011). Nevertheless, a detailed movement profile of this species is lacking, since the focus of the study was the ecophysiological advantages of paraheliotropism, thus the classification as para- or diaheliotropic leaves was based on a single measurement of leaf angle at noon.

Concerning adaptive significance, leaf movement may serve as a mechanism of optimizing light interception, thus enhancing photosynthetic productivity, or adjusting the light and/or temperature microenvironment of the leaves under normal or stress situations. In the last case, the benefit for the plant may not be productivity enhancement, but photo-protection under stress conditions. It is well documented that paraheliotropism represents an efficient, fast and reversible strategy to overcome environmental stresses such as high light and temperature (Arena et al. 2008). Additionally, paraheliotropism complements photosynthetic performance to enhance WUE and reduce the risk of photoinhibition under water stress conditions (Kao and Tsai

1998). On the other hand, diaheliotropism conforms closely with the photosynthetic response to light, concerning high-light demanding species which in several ways by-pass or overcome photoinhibition/high temperature problems (Sailaja and Rama Das 1996, Greer and Thorpe 2009).

Several hypotheses were tested for the adaptive significance of the differential and complicated leaf movement pattern of *C. spinosa*, by comparing physiological parameters of normally moving leaves with immobilized ones. Since the movement pattern shows both diaheliotropic and paraheliotropic characteristics, roles related to both types of movement were considered. It was found that the movement pattern did not offer any advantage on the daily intercepted PAR and did not enhance the performance of leaves either from a photosynthetic or from a photoinhibitory point of view. Even though considerable differences of photosynthetic and PAR characteristics may appear between clusters, the stem average values were considered as a more suitable proxy for the ascription of any movement adaptive significance. Consequently, the assessment of photosynthesis, intercepted PAR and LUE of stems on a daily basis, indicated that no advantage is offered by movement on the exploitation of higher light intensities. Another role—mainly related to paraheliotropism—is the avoidance of high light intensities, especially under co-occurring stress factors, such as—in the case of *C. spinosa*—high temperature. A potential benefit for the plant would be the avoidance of photoinhibitory conditions during midday, when high temperature (restricting the dark reactions of photosynthesis) coinciding with high light intensities might lead to overexcitation of the photosynthetic apparatus. Even though this would be a reasonable assumption for a typical plant under the stressful mid-summer Mediterranean conditions, it does not seem very likely for *C. spinosa*. Indeed, photosynthesis values as high as $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ at air temperatures over 40°C measured both in this study and in Levizou et al. (2004) can hardly indicate problems in the photosynthetic apparatus. Additionally, no amelioration was evident for either photochemical or non-photochemical quenching. Consequently, the hypothesis that leaf movement minimizes the risk of photoinhibition also had to be rejected.

Consistent with our results, other studies comparing free-moving diaheliotropic leaves with restrained ones also showed similar diurnal photosynthetic rates (Ehleringer and Hammond 1987) and no differences in the daily course of maximal PSII quantum yield (Zhang et al. 2009). Pastenes et al. (2005) found similar CO_2 assimilation rates for water-stressed free-moving and restrained paraheliotropic leaves throughout the day, but higher A_n in watered-restrained when compared with watered-unrestrained leaves. They suggested that paraheliotropism requires a cost in terms of photosynthesis, while by decreasing intercepted light and temperature it confers on the plant enhanced protective capacity against photoinhibition. With analogous results concerning photosynthesis, Bielenberg et al. (2003) concluded that the

potential loss of carbon assimilation associated with lower light intensities may be counteracted by a reduction in temperature and transpiration that leads to a substantial increase in WUE. On the contrary, Arena et al. (2008) reported a strong decrease of all measured photosynthetic characteristics of restrained paraheliotropic leaves compared with unrestrained, accompanied by an aggravated PSII photoinhibition.

A slight (but not statistically significant) amelioration was found for WUE on the moving leaves of caper, accompanied by a (marginally significant) better cooling effect, especially on stems with north–south orientation. Indeed, these stems, which present the most intense and clear moving pattern, seem to attain a better cooling effect despite the fact that they transpire less water on a daily basis. Apparently, this non-transpirational cooling is related to their movement pattern, which results in maximum intercepted PAR early in the morning (for one cluster) or in the afternoon (for the other cluster), and not during midday as in the immobilized leaves.

Better water use and enhanced cooling are obviously beneficial for a Mediterranean summergreen like *C. spinosa*, which has to cope with high ambient temperatures, reaching and surpassing 40 °C during its growth period (Levizou et al. 2004). Nevertheless, our findings are rather weak to explain the highly complicated leaf movement pattern of *C. spinosa* from an evolutionary point of view. Even though *C. spinosa* grows in Mediterranean and arid ecosystems during the summer dry period, it does not seem to encounter water limitation problems, due to its deep root system (Sozzi 2001, Rhizopoulou and Kapolas 2015), which may excrete acidic compounds, penetrating through cracks in rocks and reaching deep and well-protected water resources (Oppenheimer 1960). This is physiologically justified by the daily fluctuation of leaf water potential, which does not fall below -2.8 MPa throughout the summer period (Figure 6 and see Levizou et al. 2004). However, it has to be noted that the physiological performance of moving leaves was compared with that of immobilized ones that were stabilized for a short time period, i.e., during the afternoon before measurements. If the measurements were made after longer immobilization periods, more pronounced effects in water use or even in photosynthetic performance might have been noted.

An additional explanation for not finding considerable differences between moving and immobilized leaves may be the use of mature individuals with a well-developed root system for the measurements of the present study. Even though leaves of *C. spinosa* are produced every spring and senesce every autumn, the root system remains in the ground and is used every successive year. The question is what happens during the first year (or years) of growth after seed germination, when the root system may have not yet reached deep water resources. It is possible that during that period, leaf movement may be vital for the water economy of the young plant, until the root expands to deep

water reserves. An analogous seedling-oriented explanation was proposed by Denison et al. (2010) studying solar tracking trade-offs in alfalfa. They suggested that the photosynthetic benefits of tracking by seedlings or other low leaf area index plants (e.g., recently grazed) are sufficient to maintain diaheliotropism, despite its photosynthetic costs and season-long negative effects on community productivity.

Finally, the possibility that the moving pattern may have rendered an unknown adaptive advantage in the evolutionary past, which has diminished or disappeared in the process of the evolutionary time, cannot be excluded. In that case, under different abiotic and/or biotic conditions moving leaves might be advantageous and the pattern still remains as an evolutionary residue.

In conclusion, a novel differential diurnal leaf movement pattern with both dia- and paraheliotropic characteristics is presented for the summergreen *C. spinosa*. Even though leaf movement seems to offer better water use and enhanced cooling, the gain for the plant seems rather weak to explain the highly complicated movement pattern. Further investigation will possibly address a more reasonable explanation for the adaptive significance of this movement.

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Conflict of interest

None declared.

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