Research article

Disproportionate carbon and water maintenance costs of large corollas in hot Mediterranean ecosystems

Alberto L. Teixido [a,*], Fernando Valladares [a,b]

[a] Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Tulipán s/n, Móstoles, E-28933 Madrid, Spain
[b] Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, MNCN-CSIC, Serrano 115 dpdo, E-28006 Madrid, Spain

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ABSTRACT

Larger corollas increase the reproductive success of entomophilous plants, but are also associated with increased carbon and water costs, especially under hot and dry conditions. Minimizing floral carbon and water loss by reducing corolla size should be potentially advantageous for plants living in these environments. We quantify maintenance costs of corollas (water and carbon) in large-flowered rockroses (*Cistus* spp.) in a Mediterranean ecosystem. We performed field studies of two coflowering sympatric *Cistus* of contrasting corolla size to analyze water costs. Additionally, we used the larger-flowered species (*C. ladanifer*) to analyze the effects of intraspecific variation in corolla size on floral net carbon exchange and transpiration rates. We also assessed the mean daily percentage of plant water and carbon consumed by corollas by comparing with that of leaves at the time of flowering in *C. ladanifer*. Temperature and corolla area increased water maintenance costs, following an allometric relationship where transpiration rate per unit of area increased with corolla area. Larger flowers tended to heat less under strong irradiance than smaller ones in both species, especially in *C. ladanifer*, demonstrating a stronger transpirational cooling effect on larger flowers. In terms of carbon, temperature significantly affected net carbon exchange rates, which were not affected by corolla size. Daily water and carbon expenses of corolla were ca. 50% of those of leaves on an organ surface area basis. Our results suggest that water and carbon maintenance costs of large flowers in the Mediterranean impose significant constraints to corolla size, ecophysiologically favoring smaller-flowered individuals in these ecosystems.

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Introduction

Flower size is closely related to pollinator attraction and thus this trait plays a decisive role in the reproductive ecology of entomophilous plants. Larger corollas increase pollen deposition and removal and, as a result, increase both female and male fitness (Stanton et al., 1986; Young and Stanton, 1990; Kudoh and Wigham, 1998; Arista and Ortiz, 2007; Nattero et al., 2010). Accordingly, many studies have documented pollinator-mediated phenotypic selection toward larger corollas (e.g. Galen, 1989; Campbell et al., 1991; Conner and Rush, 1997; Totland, 2001; Nattero et al., 2010). However, since yet small-flowered plants persist in populations, Galen (1999) pointed out that a unilateral view of the evolution of flower size from a pollinator perspective was probably oversimplistic. Advantages associated with pollinator attraction can be offset by increased resource costs. For example, larger corollas are associated with greater requirements of biomass and water for floral development (Galen, 1999; Carroll et al., 2001; Elle and Hare, 2002), as well as with larger demographic costs due to water use under dry conditions (Galen, 2000).

Carbon use during flowering plays a key role in floral attraction and respiratory demands of corollas may consequently be high and even exceed the daily production of photosynthates at the whole plant (Galen et al., 1993; Vemmos and Goldwin, 1994; Lambers et al., 2008). Likewise, water is a limiting essential resource that is needed for the maintenance of corolla turgor and temperature (Galen, 2005). Temperature regulation is especially important in flowers, as thermal optima for processes contributing to sexual reproduction are narrower than optima for growth functions (Lacey, 1996; Erickson and Markhart, 2002; Young et al., 2004). Work on floral temperature has usually focused on thermogenic flowers (reviewed by Seymour, 2010) and flowers in alpine

* Corresponding author. Tel.: +34 91 488 82 90; fax: +34 91 664 74 90.
E-mail addresses: alberto.teixido@urjc.es, galegox@gmail.com (A.L. Teixido).

and subarctic climates where an increase of floral temperature has a positive effect on the development (Kevan, 1975; Stanton and Galen, 1989; Kudo, 1995; Tsukaya et al., 2002; Galen, 2006). However, floral overheating in hot climates can be damaging so transpirational cooling becomes crucial to minimize it (Patiño and Grace, 2002; Galen, 2005), whereas water shortage in dry environments can lead to an inefficient transpirational cooling. Heat and drought, acting together, can disrupt the normal performance of flowers, affecting both fruit and seed production (Konsens et al., 1991; Galen, 2000; Erickson and Markhart, 2002; Fang et al., 2010).

Mediterranean climates involve high temperatures and water and carbon shortages, imposing constraints in plant reproduction by speeding up development, shortening flowering duration (Larcher, 2000; Thompson, 2005; Aragón et al., 2008) and, occasionally, delaying the initiation of flowering until the rainy season to maximize water use efficiency (Blionis et al., 2001; Verdú et al., 2002). Minimizing floral water loss by reducing corolla size should be potentially advantageous for plants living in these environments (Galen, 2000, 2005; Elle and Hare, 2002; Herrera, 2005). However, many common Mediterranean plants such as rockroses (Cistus spp.) do exhibit large corollas. Large flowers in these hot and dry environments may suffer increased respiration and excessive evaporative demand. While there can be stomata on corollas, evidence suggests that there are too few to regulate the corolla temperature by thermoregulation (Galen et al., 1993; Patiño and Grace, 2002; Nobel, 2009). Some large-flowered plants in these and other hot and dry ecosystems show adaptations to prevent overheating and excessive water loss. Nocturnal flowering and pollination are features of several caperbushes (Capparis sp.) of semi-arid areas (Rhizopoulou et al., 2006), and most desert large-flowered cacti (Valiente-Banuet et al., 1997; Fleming et al., 2001). Floral cooling mechanisms appeared to be critical for the reproductive success of large convolvulaceous flowers in hot tropical environments (Patiño and Grace, 2002).

In this study we quantify maintenance costs of corollas in terms of water and carbon in large-flowered rockroses (Cistus spp.) in a Mediterranean ecosystem. We consider the metabolism of stored photosynthates to support petal respiration to be the main carbon costs of flowers (i.e., a negative net carbon exchange, as in Galen et al., 1993), while in other studies carbon costs of corollas were indirect costs taken as decreased photosynthetic rates of leaves at the whole plant associated to large corollas (Galen et al., 1999; Lambrecht and Dawson, 2007). A direct measurement of carbon maintenance costs of corollas gives us a precise assessment of floral maintenance costs in terms of carbon loss and allows us to compare them and evaluate them in the context of the plant. We expect that in dry Mediterranean ecosystems large corolla size coupled with a high ambient temperature involve significant costs in terms of carbon and water supplies. We hypothesize that large and numerous flowers are highly costly, not only regarding water use, but also, since corollas barely contribute to photosynthesis (Galen et al., 1993; Vemmos and Goldwin, 1994), regarding carbon for the maintenance of respiring tissues.

We performed field studies of two Cistus species (Cistaceae) to test our hypotheses. Species of Cistus are iteroparous evergreen shrubs and their disc-shaped five-petaled flowers are hermaphrodite and depend on multiple insect pollinators to set fruits (Bosch, 1992; Herrera, 1992; Talavera et al., 1993, 2001). Corolla size is positively related to intraspecific variation in pollinator visit rates, but this is not translated into a differential female fitness in the study species (Talavera et al., 2001; Arista and Ortiz, 2007). Specifically, we chose two coflowering sympatric species of contrasting corolla size, C. albidus (smaller) and C. ladanifer (larger), as a reference system to analyze water costs. C. ladanifer's flowers are one of the largest in the Mediterranean (Arrington and Kubitzki,

2003), reaching up to approx. 11 cm in diameter (Teixido et al., 2011). Additionally, we used C. ladanifer to analyze the effects of intraspecific variation in corolla size on floral transpiration and net carbon exchange rates. High within-individual variation in corolla size in this species (48%: A.L. Teixido, personal observations) creates an opportunity to test whether the physiological costs of large flowers vary not only among species but also among individual flowers within a population and even within an individual plant.

Materials and methods

Study system

C. albidus L. and C. ladanifer L. (Cistaceae) are related species with contrasting flower size (mean diameter ± SD: 5.30 ± 0.49 vs 9.19 ± 0.77 cm, respectively, $n = 42$). C. albidus is a shrub 40–100 cm tall that inhabits calcareous and dry soils. Flowering phenology spans from February to June and each plant produces purplish-pink flowers in terminal heads pollinated by beetles and bees (Bosch, 1992; Muñoz-Garmendía and Navarro, 1993). C. ladanifer is a shrub 100–250 cm tall that inhabits acidic and dry soils. Flowering phenology spans from March to June and each plant produces solitary white flowers often exhibiting dark colored spots at their bases (Muñoz-Garmendía and Navarro, 1993). Flowers are mainly pollinated by bees, beetles and flies (Talavera et al., 1993). Both C. albidus and C. ladanifer are self-incompatible, but self-pollinated flowers of the former species can set some seeds (Bosch, 1992; Herrera, 1992). Flowers open synchronously each morning within populations and lose their petals in the afternoon.

The study was conducted between April and May of 2011 in the Madrid province, central Spain (39°53′–41°09′ N, 3°03′–4°34′ W). Two different sites were utilized to study floral maintenance costs in terms of water and carbon. Due to volume of work, we could not carry out both measurements at the same site. However, our objective and subsequent results were not affected by this matter since we aimed to evaluate the effects of corolla size and air temperature on floral maintenance costs so we have worked on the very same area and study system (i.e., large flowers under stressful conditions), ensuring that plants and environmental conditions during flowering peak were almost identical. Both differences in floral transpiration rates between coflowering sympatric species and between sunny and cloudy days in C. ladanifer were monitored in San Agustín de Guadalix (740 m a.s.l.; 40°41′ N 3°36′ W; hereafter Pop1-transpiration) between April and May. The area is on a limestone and gypsum soil with granitic outcrops and is covered by an open scrubland vegetation on a south facing hilly slope. Climate is dry, with an annual mean precipitation of 567 mm and an annual mean temperature of 13 °C (Ninyerola et al., 2005). Floral net carbon exchange rates in C. ladanifer were monitored in Becerril de la Sierra (1120 m a.s.l.; 40°44′ N 3°57′ W; hereafter Pop2-net carbon exchange) in May. This area is on a granite soil covered by sparse trees on a south facing slope. Climate is subhumid, with an annual mean precipitation of 820 mm and an annual mean temperature of 11 °C (Ninyerola et al., 2005). We are aware that conditions at both sites clearly differed but we focused on the main objective of the study: quantifying maintenance costs of corollas in terms of water and carbon in large-flowered species in a Mediterranean environment, independent from the population context.

Microclimate was recorded during the study period at each population to relate it to floral maintenance costs. Microclimatic variables measured were air temperature (°C), air relative humidity (%), soil moisture (%) and solar irradiance (μmol PAR m^{-2} s^{-1}). We also calculated VPD from air temperature and relative humidity. Sensors were used for air temperature and relative humidity

(Hobo H08-032-08, Onset, Pocasset, MA, USA) located 1 m above the ground. Readings of each sensor were recorded every 15 min with a Hobo H08-006-04 data-logger. Soil moisture was recorded at 3–5 points below the canopy in the understorey of each plant with sensors recently calibrated based on integrated TDR technology (ECH$_2$O EC-20, Decagon Devices, Pullman, WA, USA). Solar irradiance was recorded every 30 min with a quantum sensor (Apogee quantum sensor QSO-SUN, Logan, UT, USA). At each site, all microclimatic variables were significantly correlated each other (Table S1). Air temperature was positively correlated with solar irradiance and negatively correlated to air relative humidity and soil moisture. As a consequence, we only used air temperature as predictor variable to explain floral maintenance costs.

Additionally, we recorded floral temperature of *C. albidus* and *C. ladanifer* in Pop1-transpiration to evaluate whether that temperature is higher or lower than air temperature and whether it follows a predictable pattern regarding corolla size. Since floral temperature is likely to be influenced by both air temperature and corolla size we corrected this matter by comparing floral temperature with temperature of a reference surface (Jones, 2004). We used a standard reference system so that any researcher can subsequently work with our absolute data. Any variation in floral temperature due to differences in environment conditions, between species and/or populations are deleted by means of this reference system. We used a wet white filter paper to evaluate differences between floral temperature and a filter paper temperature as a standard surface exposed to evaporation (Jones, 2004). The effect of corolla size on floral temperature is affected by transpiration, which in turn is affected by boundary layer resistance. A positive relationship between corolla size and floral temperature indicates the importance of the boundary layer of large flowers to increasing floral transpiration rates. A negative relation would indicate a higher floral transpirational cooling in large flowers due to higher water uptake. To address all these assumptions filter paper was wet early in the morning before measuring floral temperature and transpiration rates. Filter paper was not rewetted but it remained wet during the measurements. Then we recorded filter paper temperature every 30 min and floral temperature of every flower using an infrared thermal Camera (FLIR B2 IR Thermal Imager, 2006; FLIR Systems, Inc, Willsonville, OR, USA) right before measuring corolla size and transpiration rates. All images were subsequently analyzed in ThermaCam QuickView v1.3 (FLIR Systems, Inc, 2005; Willsonville, OR, USA). We also calculated flower-to-air VPD from floral temperature and atmospheric measurements.

Effects of temperature and corolla size on floral transpiration rates and temperature

We carried out two different observational designs to evaluate the effects of temperature and corolla size on floral transpiration rates. First, we examined differences in floral transpiration between the coflowering sympatric *C. albidus* and *C. ladanifer*. During the coflowering peak in mid-April of 2011, we selected 21 adult plants per species. At each plant, we selected two mature, healthy, fully-expanded flowers of contrasting size and on different terminal branches to record within-individual variation. Since the flowers of these species lack solar tracking (i.e. heliotropism), all flowers were carefully selected to be sun-oriented. All plants and their paired-flowers were measured only once. We measured three plants per species and per day between 08:00 am and 14:00 pm, in one plant of either of the species every hour during seven consecutive days. We alternated daily the species every hour and carried out the opposite pattern at the following day, so each species was measured at least three times per hour on three different days. Between those hours all flowers were completely open and after 14:00 pm petals

started falling off. On a petal of every flower we recorded stomatal conductance to water vapor, g_s (mmol m^{-2} s^{-1}), with a portable steady-state porometer (Model SC-1, Decagon Devices, Pullman, WA, USA). Since this porometer only measures fluxes from one side of the organ measured, we recorded conductance on the abaxial surface. Petals are known to have none or only few stomata so water loss regulation is very limited in corollas and largely depends on their cuticle physics (Galen et al., 1993; Patiño and Grace, 2002; Nobel, 2009). We assumed that both surfaces have the same conductances and we measured the abaxial surface only so data could be directly compared to those of the leaves. Air and floral temperature and the other microclimatic variable data were recorded as described above (see "Study system"). Then floral transpiration rate on a surface area basis, E_f (g H$_2$O m^{-2} h^{-1}), was calculated from air and floral temperature, relative humidity and stomatal conductance to water vapor (g_s) data (see the Supporting Information presented in Methods for further details).

As fully-expanded organs in plants mainly lose water through their outer epidermis, corolla surface area (cm^2) was used to test for relationships between corolla size and water use after flower growth has ceased. We recorded corolla diameter (cm) after transpiration rates were taken on each paired-flowers. On the first 20 flowers measured per species we also excised one petal per flower. Excised petals were carefully unfolded and photographed on a black sheet to assess their surface area by means of image processing in ImageJ v1.43 (ImageJ, 2010; US National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/). Then we multiplied each value by five to estimate corolla surface area, since flowers have five petals. Corolla surface area and corolla diameter were closely correlated in both species (Pearson's $r = 0.912$, $P < 0.001$, $n = 20$ for *C. albidus*; Pearson's $r = 0.837$, $P < 0.001$, $n = 20$ for *C. ladanifer*). Then we indirectly estimated corolla surface area from corolla diameter for the remaining flowers by means of each given regression equation.

Second, we examined differences in floral transpiration rates depending on among and within-individual variation in corolla size and temperature variation between sunny and cloudy days only in *C. ladanifer*. During the flowering peak between mid-April and early May we worked on 35 similar adult individuals. We used the 21 plants of *C. ladanifer* used in the differences in floral transpiration rates with *C. albidus* and, additionally, we selected 14 new plants in the same population. Overall, 18 plants were measured in sunny days and 17 in cloudy ones. At each plant, we selected a pair of mature, healthy, fully-expanded flowers of contrasting size on the same terminal branch to record within-individual variation. Sun orientation followed the same criteria as described above even for cloudy days. All plants and their paired-flowers were measured only once between 10:00 am and 13:00 pm when the flowers were fully-expanded. All measurements including stomatal conductance to water vapor, floral temperature, microclimatic variables, transpiration rates and corolla size were recorded as described above.

Effects of temperature and corolla size on floral net carbon exchange

During the flowering peak in late May of 2011 in Pop2-net carbon exchange, we selected 20 similar adult individuals of *C. ladanifer*. Criteria of paired-flower selection and sun orientation were followed as described above. During six study days all plants and their paired-flowers were measured only one time between 10:00 am and 13:00 pm. On a petal of every flower we recorded petal net carbon exchange, NCE$_f$ (μmol CO$_2$ m^{-2} s^{-1}), using a LI-6400 infrared gas analyser. NCE$_f$ was measured at constant and ambient CO$_2$ concentration (400 μmol CO$_2$ mol^{-1}) using the built-in LI-6400 CO$_2$ controller, and saturating light intensity

($1500 \, \mu mol \, PAR \, m^{-2} \, s^{-1}$) provided by the built-in LI-6400 blue-red light source. Monitored microclimatic variables (air temperature and relative humidity) were kept constant and close to ambient conditions each day. The portable steady-state porometer and the LI-6400 infrared gas analyser were cross-calibrated and the correlation of the respective measurements was very high (>0.95). Temperature and humidity conditions inside the chambers were checked and matched with ambient conditions, and were calibrated daily. We recorded net carbon exchange on the abaxial surface following the same criteria followed for conductance. All remaining measurements including floral temperature, microclimatic variables and corolla size were recorded as described above. We tried to measure gas exchange of flowers under the same air temperature range and humidity as that taking place during the floral transpiration determinations. The match was not perfect because during the flowering period in Pop2-net carbon exchange ambient conditions were more homogeneous than during the period of maximum flowering in Pop1-transpiration. This slight mismatch must be taken into account when interpreting our results.

Water and carbon maintenance costs of flowering

Comparisons of daily floral transpiration rates between *C. albidus* and *C. ladanifer* were made per flower and plant. At each species, we assessed the mean total area that plants displayed in corollas ($m^2 \pm SD$) and the mean E_f ($g \, H_2O \, m^{-2} \, h^{-1} \pm SD$). Then we recorded the mean daily E_f per flower ($g \, H_2O \, flower^{-1} \, d^{-1} \pm SD$) by considering the 6 h (08:00 am to 14:00 pm) that flowers were open and functional. Despite the dependence of flower transpiration on air temperature (see Results) and temperature increases from 08:00 am to 14:00 pm, our measurement of mean daily E_f per flower involved the same number of flowers measured every hour ($N = 6$ or 8), between 08:00 am and 14:00 pm. To calculate the mean daily E_f per plant ($g \, H_2O \, plant^{-1} \, d^{-1} \pm SD$) we recorded the mean number of open flowers of each species. It is worth noting that each mean daily E_f per plant is a representative mean for each species, providing an integrated value of how much water and carbon, on average, a plant of each species uses. Additionally, each mean per species took into consideration the intraspecific variation in corolla size and number of open flowers.

We also assessed the mean daily percentage of plant water and carbon consumed by corollas by comparing with that of leaves at the time of flowering in *C. ladanifer*. To evaluate floral costs in the context of the plant, data of leaf transpiration, E_l ($mol \, H_2O \, m^{-2} \, d^{-1}$), leaf respiration, R_l ($\mu mol \, CO_2 \, m^{-2} \, s^{-1}$), and leaf area index, LAI ($m^2 \, m^{-2}$), were taken from the same plants and under the same environmental conditions using the same equipment and sampling scheme. We first measured gas exchange of the leaves of the species and then we compared maintenance costs of flowering with maintenance costs of vegetative structures.

To evaluate daily water cost experienced by flowering in *C. ladanifer*, floral transpiration rates were compared to leaf transpiration rates. We measured the whole plant area (m^2) – as the area of an ellipse (πab), the most proximate area for this species' shape– of all 21 individuals used in the study of floral transpiration between *C. albidus* and *C. ladanifer*. We also measured leaf area index (LAI) for both species. LAI was destructively determined in a subsample of the crown by collecting all the leaves included on a quadrangular prism of $0.4 \, m^2$ of basal area; i.e. we established a quadrangular prism with its $0.4 \, m^2$ on the ground and collected all crown material that was confined within it. Leaves were then scanned to calculate total leaf area corresponding to 0.4 m of

ground to obtain LAI and then calculated total leaf area per plant ($m^2 \, plant^{-1}$) multiplying mean LAI by the projected area of the crown. Projected area of the crown was determined by measuring the area of the silhouette resulting from the vertical projection of the crown on the soil. The method was checked for instrumental error and we estimated it to be less than 3%. Subsequently, from mean E_l ($g \, H_2O \, m^{-2} \, h^{-1} \pm SD$) we recorded mean daily E_l per plant ($g \, H_2O \, plant^{-1} \, d^{-1} \pm SD$). We included 12 h of leaf transpiration. We then compared mean daily E_f (as calculated above) with mean daily E_l and assessed the percentage of daily water expenditure by flowers. Furthermore, we evaluated the role of flowers in plant water status by considering how important daily water cost of flowering is relative to the area of corollas vs that of leaves. Thus, we recorded the mean floral display area ($m^2 \pm SD$), by multiplying the mean total area that plants displayed in corollas by mean number of open flowers and next we compared the daily water cost of corollas relative to their overall area.

To evaluate carbon cost involved in *C. ladanifer* flowering, floral net carbon exchange rates were compared to leaf respiration rates. We did this comparison since we recorded carbon loss in flowers, i.e., the exchange was negative due to petal respiratory demands (see Results). Conversely to the net carbon exchange measurements made in flowers at saturating light, we measured respiration in the dark on leaves. Dark conditions were simulated by covering leaves with aluminum foil and then respiration was recorded after 10 min after of dark adaptation as in Zaragoza-Castells et al. (2008). In this case, we could not compare daily respiration rates since we only recorded measurements in flowers between 10:00 am and 13:00 pm, so we compared values during the hours of highest floral activity. Therefore, this involves that our comparisons between flowers and leaves in terms of carbon cost were made only during these hours instead of daily. We also measured both the whole plant area (m^2) and the leaf area per plant ($m^2 \, plant^{-1}$) of all 20 individuals in Pop2-net carbon exchange as described above. Subsequently, from mean R_l ($\mu mol \, CO_2 \, m^{-2} \, s^{-1} \pm SD$) we recorded the mean R_l per plant ($\mu mol \, CO_2 \, plant^{-1} \, s^{-1} \pm SD$). Then we calculated the mean NCE_f per plant ($\mu mol \, CO_2 \, plant^{-1} \, s^{-1} \pm SD$) by considering the mean NCE_f per flower ($\mu mol \, CO_2 \, flower^{-1} \, s^{-1} \pm SD$) and the mean number of open flowers. Thus, we compared both the mean instantaneous NCE_f and the mean R_l per plant and recorded the percentage of carbon cost of flowering. We also evaluated the impact of flowers in plant carbon balance by considering how important carbon cost of flowering is relative to the area that corollas cover against leaf area. This step was carried out as described above for daily water cost of corollas, but we compared instantaneous carbon cost of corollas in s^{-1} instead of d^{-1}. Overall, floral costs in the context of the plant were carried out and showed in the context of species and the comparisons were corresponding with each population where we measured each rate.

Statistical analysis

To determine the significance in floral transpiration rates between coflowering species differing in flower size, we fitted a General-linear model ANCOVA, considering species (fixed factor), plant (random factor nested within species) and air temperature (covariate; fixed factor) as independent variables, and the interaction Species × Air temperature. Since our data set had a nested structure and our experimental units (flowers) were not fully independent, we considered variation within plants by including plant within species as a random factor in our model. In this way, we included a statistical control of this source of variation and, additionally, including the term plant nested in species we are partialling out this effect and then we can focus on the variation of interest: species variation, air temperature variation and

the interaction between these two fixed predictors (Littell et al., 1996; Quinn and Keough, 2002). The assumptions of normality and homogeneity of variance were tested using Shapiro–Wilk's and Levene's tests, respectively. Within-individual variation in floral transpiration rates between paired-flowers with contrasting size was analyzed by means of a paired *t*-test for each species.

To test differences in floral temperature between species, air temperature and corolla size, we used the difference between floral temperature and filter paper temperature as our response variable to correct the likely effect of the relation between air temperature and corolla size within-species in floral temperature. The assumptions of normality and homogeneity of variance were tested as described above. Then we first fitted a General-linear model ANCOVA as also described above for transpiration, including the same variables. Second, we evaluated the effects of corolla size on floral temperature by conducting two different linear regressions, one for each species.

To determine the significance in floral transpiration rates between sunny and cloudy days in *C. ladanifer*, we fitted a new General-linear model ANCOVA, considering day (fixed factor), plant (random factor nested within day), corolla size (covariate; fixed factor) and the interaction species × corolla size as independent variables. The assumptions of normality and homogeneity of variance were tested using Shapiro–Wilk's and Levene's tests, respectively. Variations in corolla size and floral transpiration rates between paired-flowers within-individuals were analyzed by means of paired *t*-tests while variations in corolla size, air temperature and air relative humidity between sunny and cloudy days were analyzed by means of Student's *t*-tests.

To test the effects of corolla size and air temperature on floral net carbon exchange rates in *C. ladanifer* we fitted a regression linear analysis. Since both predictive variables were not correlated with each other ($r = 0.192$, $P = 0.235$, $n = 40$), they were included together in a multivariate regression model. The assumptions of normality and homogeneity of variance to meet assumptions of regression were tested using Shapiro–Wilk's and Levene's tests, respectively. Within-individual variation in floral net carbon exchange rates between paired-flowers with contrasting size was analyzed by means of a paired *t*-test.

ANCOVAs, paired *t*-test and regression analyses were performed using the R statistical package version 2.12.1 (R Development Core Team, 2010).

Results

Effects of air temperature and corolla size on floral transpiration rates and temperature

Larger corollas of *C. ladanifer* vs *C. albidus* translated into significantly higher transpiration rates (mean ± SD: 64.1 ± 45.6 and

Table 1

Results of ANCOVA for effect of species, air temperature and the interaction between species and air temperature on corolla transpiration rates of *C. albidus* and *C. ladanifer*. $N = 21$ plants per species. Significant *P*-values are marked in bold.

Effect	df	MS	F	P
Species	1	3002.1	56.7	**<0.001**
Plant (species)	19	1024.6	4.02	**0.024**
Air temperature	1	6089.5	102.4	**<0.001**
Species × Air temperature	1	3985.9	87.1	**<0.001**
Error	80	35.4		

17.6 ± 6.4 g H_2O m^{-2} h^{-1}, respectively; Table 1). The influence of corolla size on floral transpiration was allometric since transpiration rate per unit of area increased with corolla area. Thus, larger flowers entail disproportionate water costs not only for being larger but because they transpire more per unit of area. Air temperature significantly affected floral transpiration rates but this effect differed depending on the species (Species × Air temperature significant, Table 1). The higher the temperature the higher the transpiration in both species ($R^2 = 0.28$, $P = 0.02$, $n = 42$ for *C. albidus*; $R^2 = 0.83$, $P < 0.01$, $n = 42$ for *C. ladanifer*), with similar floral transpiration rates at lower temperatures in the two species, but with exponentially increasing rates in the larger-flowered species *C. ladanifer* at higher temperature (Fig. 1A). These results were similar when using VPD instead of air temperature and flower-to-air VPD for each species (Fig. 1B and C). Corolla size significantly differed between paired-flowers within-individuals for each species (mean ± SD: 22.0 ± 5.3 vs 18.2 ± 5.1, paired *t*-test$_{1,20}$ = 8.3, $P < 0.01$ for *C. albidus*; mean ± SD: 59.8 ± 8.9 vs 53.5 ± 8.0, paired *t*-test$_{1,20}$ = 9.0, $P < 0.01$ for *C. ladanifer*). According to this, floral transpiration rates also did, significantly increasing in larger corollas (mean ± SD: 10.7 ± 4.6 vs 9.5 ± 4.1, paired *t*-test$_{1,20}$ = 4.8, $P = 0.02$ for larger and smaller corollas, respectively, in *C. albidus*; mean ± SD: 36.6 ± 14.4 vs 33.2 ± 12.9, paired *t*-test$_{1,20}$ = 5.6, $P = 0.01$ for larger and smaller corollas, respectively, in *C. ladanifer*) significantly differed between paired-flowers within-individuals for each species.

Floral temperature averaged 26.9 ± 4.2 °C for *C. albidus* and 26.5 ± 5.4 °C for *C. ladanifer*. Air temperature averaged 23.4 ± 3.4 °C and filter paper temperature averaged 15.4 ± 2.2 °C. Flowers were significantly hotter than air (paired *t*-test$_{1,79}$ = 7.3, $P < 0.01$) and, on average, 11.0 ± 3.6 °C hotter than filter paper. Differences between floral temperature and filter paper temperature used in our analysis did not significantly differ between species (Table 2), so interspecific differences in corolla size did not affect floral temperature. In fact, flower-to-air VPD between species was also similar (mean ± SD: 2.4 ± 0.5 and 2.3 ± 0.8 for *C. albidus* and *C. ladanifer*, respectively; $F_{1,81} = 2.7$, $P = 0.11$). The only effect on floral temperature was air temperature, which similarly affected both species (Species × Air temperature not significant, Table 2).
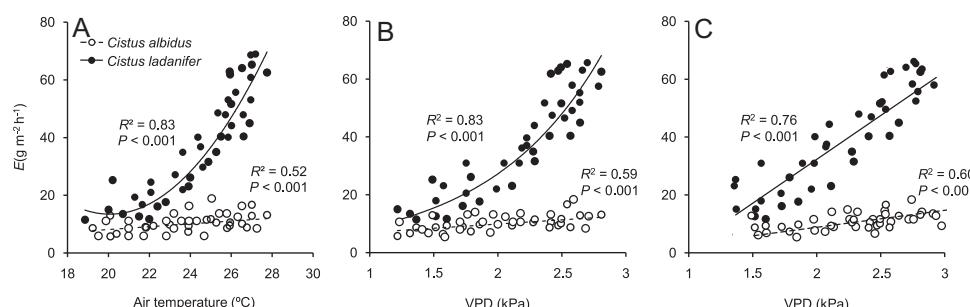
Fig. 1. Corolla transpiration rates (E) vs (A) air temperature, (B) VPD and (C) flower-to-air VPD in the two coflowering sympatric rockroses *Cistus albidus* and *Cistus ladanifer*. Each point represents one flower, with two flowers per plant and species. $N = 21$ plants per species.

Table 2
Results of ANCOVA for effect of species, air temperature and the interaction between species and air temperature on differences between floral temperature of *C. albidus* and *C. ladanifer* and filter paper temperature. $N = 21$ plants per species. Significant *P*-values are marked in bold.

Effect	df	MS	F	P
Species	1	0.9	0.3	0.725
Plant (species)	19	1.2	0.2	0.711
Air temperature	1	45.1	7.0	**0.019**
Species × Air temperature	1	0.8	0.3	0.822
Error	80	5.9		

Intraspecific variation in corolla size did not explain differences in floral temperature among-individuals either, but larger flowers tended to heat less than smaller ones in both species, especially in *C. ladanifer* ($R^2 = 0.06$, $\beta = -0.10 \pm 0.06$, $F_{1,20} = 2.0$, $P = 0.11$ for *C. albidus*; $R^2 = 0.10$, $\beta = -0.07 \pm 0.04$, $F_{1,20} = 2.8$, $P = 0.06$ for *C. ladanifer*), demonstrating a stronger transpirational cooling effect on larger flowers, matching the results reported in Fig. 1A (higher floral transpiration rates in *C. ladanifer* than in *C. albidus* under high temperatures) and the allometry between corolla size and floral transpiration rates.

For the intraspecific study in *C. ladanifer*, corolla size significantly differed between paired-flowers within-individuals (mean ± SD: 58.9 ± 8.6 vs 52.3 ± 8.2 cm^2, respectively; paired *t*-test$_{1,34} = 11.0$, $P < 0.01$), but did not between sunny and cloudy days (mean ± SD: 56.8 ± 9.9 vs 54.5 ± 8.0 cm^2, respectively; Student's *t*-test$_{1,68} = 1.1$, $P = 0.29$). In cloudy days, air temperature was significantly lower (mean ± SD: 21.0 ± 1.9 vs $26.6 \pm 1.3\,°C$ respectively; Student's *t*-test$_{1,68} = -15.0$, $P < 0.01$) and air relative humidity significantly higher (mean ± SD: 42.2 ± 4.1 vs $28.6 \pm 3.8\%$, respectively; Student's *t*-test$_{1,68} = 17.0$, $P < 0.01$). The study also showed significant differences in floral transpiration rates between sunny and cloudy days (Table 3). On average, in sunny days water loss from corollas was 78.7 ± 6.4 g H$_2$O m^{-2} h^{-1}, about four-fold more than during cloudy days, when corollas transpired 18.6 ± 12.8 g H$_2$O m^{-2} h^{-1}. Both the effect of corolla size and its interaction with the type of day on floral transpiration rates were marginally significant (Table 3). Larger-flowered individuals tended to spend more water in sunny days (Fig. 2). Within-individual variation in corolla size also significantly affected floral transpiration rates (paired *t*-test$_{1,34} = 4.52$, $P < 0.001$). Between paired-flowers differing in corolla size larger flowers transpired, on average, 72.4 ± 54.8 g H$_2$O m^{-2} h^{-1} whereas smaller flowers transpired 63.3 ± 47.2 g H$_2$O m^{-2} h^{-1}.

Effects of air temperature and corolla size on floral net carbon exchange rates

Net carbon exchange rate of corollas of *C. ladanifer* averaged -0.9 ± 0.4 μmol CO$_2$ m^{-2} s^{-1}. Therefore, there was carbon loss since net carbon exchange was negative. Overall, air temperature and corolla size explained 19% of variation in net carbon exchange

Table 3
Results of ANCOVA for effect of the type of day (sunny vs cloudy), corolla size and the interaction between day and corolla size on corolla transpiration rates of *C. ladanifer*. $N = 35$ plants. Significant *P*-values are marked in bold.

Effect	df	MS	F	P
Day	1	1123.8	11.8	**<0.001**
Plant (day)	19	765.5	2.8	**0.030**
Corolla size	1	180.2	2.7	0.056
Day × Corolla size	1	511.0	2.4	0.073
Error	66	0.3		
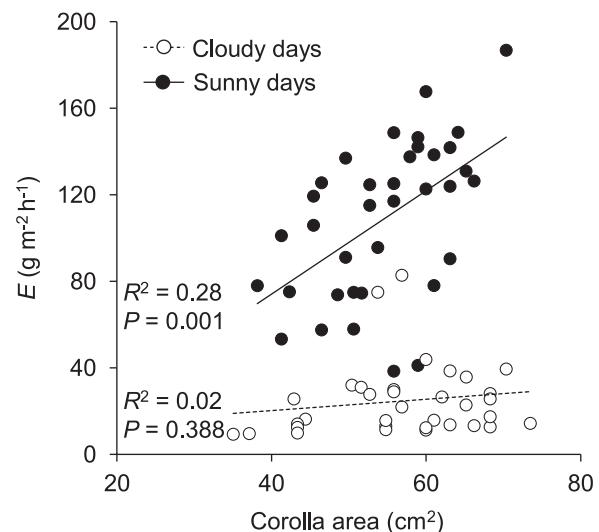
Fig. 2. Interaction between type of day (cloudy vs sunny) and corolla area in corolla transpiration rates in *Cistus ladanifer*. Corolla size and floral transpiration rates of paired-flowers at every plant were averaged for the regression-based analyses so that the unit of replication is the plant. Each point represents one flower, with two flowers per plant. $N = 18$ plants for sunny days and 17 plants for cloudy days.

($R^2 = 0.19$, $F_{2,19} = 2.3$, $P = 0.10$). This was especially due to air temperature, which showed a marginal significance in the whole model ($\beta = -0.088 \pm 0.029$, $P = 0.06$). Air temperature was positively related to floral net carbon exchange rates (Fig. 3), but coefficient β was negative because CO$_2$ exchange was loss due to petal respiration. Corolla size itself was not significant ($\beta = -0.008 \pm 0.007$, $P = 0.27$), indicating that there is not an allometric relationship with floral net carbon exchange rates. Corolla size significantly differed between paired-flowers within-individuals (mean ± SD: 56.5 ± 9.3 vs 49.3 ± 9.0 cm^2, respectively; paired *t*-test$_{1,19} = 6.3$, $P < 0.01$), but within-individual variation in net carbon exchange did not differ between paired-flowers with contrasting size (mean ± SD: -1.0 ± 0.4 vs -0.9 ± 0.4 μmol CO$_2$ m^{-2} s^{-1}, for large and small flowers, respectively; paired *t*-test$_{1,19} = -1.8$, $P = 0.08$). However, larger-flowered individuals did involve increased carbon costs per flower (μmol CO$_2$ flower^{-1} h^{-1}; $R^2 = 0.3$, $F_{1,19} = 8.4$, $P = 0.01$).
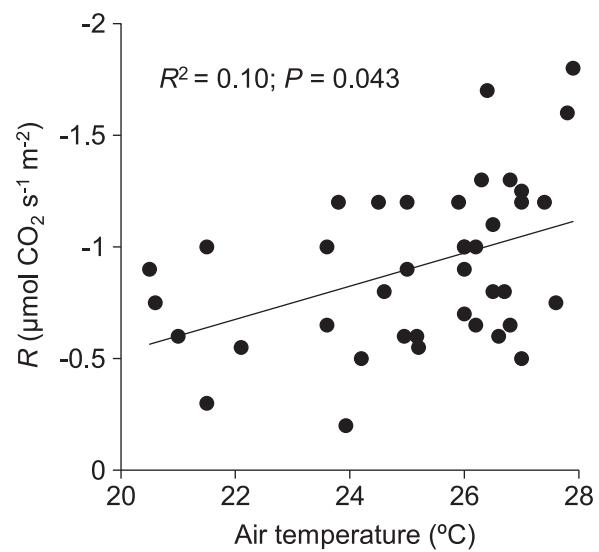
Fig. 3. Corolla respiration rates vs air temperature in *Cistus ladanifer*. Each point represents one flower, with two flowers per plant. $N = 20$ plants.

Table 4

Water and carbon maintenance costs of flowering in comparison with that of leaves in *C. ladanifer*. LAI and mean plant area of each population are provided. Mean transpiration rates (E) were only estimated in Pop1-transpiration and mean respiration rates (R) were only estimated in Pop2-net carbon exchange. $N = 21$ plants for Pop1-transpiration and 20 plants for the Pop2-net carbon exchange.

	Pop1-transpiration				Pop2-net carbon exchange			
	LAI (m² leaf m⁻²)	Mean area (m² plant⁻¹)	Mean E (g m⁻² h⁻¹)	Mean E plant⁻¹ d⁻¹ (g plant⁻¹ d⁻¹)	LAI (m² leaf m⁻²)	Mean area (m² plant⁻¹)	Mean R (μmol m⁻² s⁻¹)	Mean R plant⁻¹ d⁻¹ (μmol plant⁻¹ s⁻¹)
Corollas	–	0.17 ± 0.02	64.13 ± 45.58	56.67 ± 41.73	–	0.15 ± 0.03	−0.91 ± 0.24	−0.14 ± 0.07
Leaves	0.94	1.43 ± 0.96	29.26 ± 16.25	1003.54 ± 671.36	1.50	2.89 ± 0.91	−1.42 ± 0.47	−4.33 ± 1.37

Water and carbon maintenance costs of flowering

On average, one flower of *C. albidus* transpired $0.2 \pm 0.1\,\text{g}\,H_2O\,d^{-1}$ whereas one flower of *C. ladanifer* transpired $1.9 \pm 1.4\,\text{g}\,H_2O\,d^{-1}$. Thus, water costs of floral transpiration increased ten-fold in the larger-flowered species *C. ladanifer*. When considering plant daily flowering this relationship slightly decreased since, on average, *C. albidus* displayed more flowers than *C. ladanifer* (mean ± SD: 39.6 ± 37.7 vs 29.2 ± 16.7 daily open flowers, respectively). Overall, daily flowering involved $7.6 \pm 2.4\,\text{g}\,H_2O\,\text{plant}^{-1}\,d^{-1}$ in *C. albidus* vs $56.7 \pm 41.7\,\text{g}\,H_2O\,\text{plant}^{-1}\,d^{-1}$ in *C. ladanifer*.

Water and carbon maintenance costs of corollas of *C. ladanifer* relative to that of the foliage of the plant are shown in Table 4. Instantaneous transpiration rates of corollas were more than two times higher than those of leaves on a surface area basis of the organ and per time unit. In terms of carbon, respiration rates of corollas reached up to 64% of leaf respiration rates per unit of organ area and time and per time unit. Considering the total floral display and leaf surface area of the plant, transpiration and respiration of corollas accounted for 5.7% and 3.2%, respectively, of the whole water and carbon spent daily for the maintenance of the aboveground part of the plant, excluding stem respiration (see "Mean E plant⁻¹ d⁻¹" and "Mean R plant⁻¹ d⁻¹" between corollas and leaves in Table 4, respectively). However, when considering the percentage of floral display mean area on the plant total area (about 12% and 5% in Pop1-transpiration and Pop2-net carbon exchange, respectively; to compare "Mean area" between corollas and leaves in Table 4), corollas transpire and respire ca. 50% than leaves on an organ surface area basis (47.5 and 62.2%, respectively). This value is noticeable because corollas remain open and functional only about 6 h.

Discussion

Our study demonstrates the importance of corolla size and air temperature on floral maintenance costs in large-flowered plants in a Mediterranean environment. We found significant species differences on transpiration rates of corollas in natural populations of the coflowering sympatric *C. albidus* and *C. ladanifer* differing in flower size. We also found that these transpiration rates strongly increased with air temperature in the larger-flowered species *C. ladanifer*. In agreement with this we found evidence for an allometric relationship between size and transpiration of corollas. Furthermore, we detected a reduced overheating in larger corollas with increasing air temperature indicating that corolla size affects transpirational cooling (i.e., water used as a thermoregulator mechanism). However, transpiration in corollas did not counteract the effect of radiation since floral temperature was always higher than air temperature. We also found a significant effect of air temperature, but not of corolla size, on floral net carbon exchange rates on corolla surface area basis in *C. ladanifer*. Although we cannot

discard a minimal photosynthetic capacity of petals, our negative values of floral net carbon exchange imply significantly higher petal respiratory demands than photosynthetic gains. Overall, we estimated that 1 m² of corollas used about half of the water and carbon spent by 1 m² of foliage of a plant in our study populations. Our results indicate that both water and carbon losses by functional corollas are elevated, particularly in large-flowered Mediterranean species.

Water costs of corollas

Previous work has revealed that there is an increase in transpiration associated with flowering, thus making flowering particularly expensive in the use of water (Nobel, 1977a; Whiley et al., 1988; Galen et al., 1999; Galen, 2006). The relevance of water spent by transpiration in corollas is dependent, among other factors, on their size and on the environmental conditions. Galen et al. (1999) reported a positive relationship between corolla area and rates of water uptake by fully-expanded flowers in the alpine wildflower *Polemonium viscosum* (mean: $0.024\,\text{g}\,H_2O\,\text{flower}^{-1}\,h^{-1}$). Other studies have reported higher rates of water loss in corollas of large-flowered trees in tropical dry forests flowering during the dry season (mean rates: 0.05–$0.115\,\text{g}\,H_2O\,\text{flower}^{-1}\,h^{-1}$; Chapotin et al., 2003) and of large-flowered cacti in California (mean rates: 0.07–$0.46\,\text{g}\,H_2O\,\text{flower}^{-1}\,h^{-1}$; Nobel and De la Barrera, 2000; De la Barrera and Nobel, 2004a; E. De la Barrera, personal communication). Mean floral transpiration rate in *C. albidus* found here ($0.03\,\text{g}\,H_2O\,\text{flower}^{-1}\,h^{-1}$) was within the lower range of previous reports, while that of *C. ladanifer* ($0.32\,\text{g}\,H_2O\,\text{flower}^{-1}\,h^{-1}$) was within the highest range reported previously.

One other aspect of differences in transpiration to be considered is flower color, due to the relation of this trait to thermal biology (reviewed in Willmer, 2011). Dark-colored flowers are particularly absorptive of solar radiation and can therefore warm up well above ambient temperatures (Patiño and Grace, 2002; Sapir et al., 2006). Whether floral evaporative cooling could be stronger and avoid a higher overheating in dark-colored flowers remains unclear, but it would not be unexpected in hot climates. However, we did not find any difference in floral temperature between purplish-pink (*C. albidus*) and white (*C. ladanifer*) flowers and, further, we reported that white, larger flowers, increased transpiration rate per unit of area. We can conclude that, in our study system, flower size is relevant in water maintenance floral costs, but further observations of floral temperature and cooling due to flower color and size are much needed.

The high rates of water loss by corollas of *C. ladanifer* indicate the high potential for transpirational cooling of these large corollas in the Mediterranean. In agreement with the allometric relationship reported in our study, Galen (2006) found that *Ranunculus adoneus* reduced excess temperature in solar tracking flowers, but not in non-tracking flowers at a water cost (transpiration). We did not find any effect of the species on floral temperature between *C. albidus*

and *C. ladanifer* despite their contrasting flower size, suggesting that transpirational cooling was more effective in *C. ladanifer*. This result matches with the allometric pattern between corolla size and floral transpiration rates and with the higher water costs in this species. Additionally, this efficiency may explain the high rates of water loss reported for *C. ladanifer* under higher temperatures, which prevented overheating. In contrast, Patiño and Grace (2002) reported that the species with larger corollas developed higher excess temperatures than those of medium and small-sized corollas among tropical convolvulaceous flowers.

Despite that large corollas appear to be expensive in terms of water, our reference system (filter paper) revealed that their transpiration rates were not maximal, suggesting constraints in floral transpiration. This limited transpiration can influence two essential aspects of the reproductive ecology of these plants. First, limitation of water loss because of cuticle characteristics of the corolla improves the water economy of the whole plant, especially important in a Mediterranean environment (Thompson, 2005). Second, temperature increase may provide a better microclimate for visiting insects (Dyer et al., 2006; Sapir et al., 2006; Rands and Whitney, 2008), although the primary benefit of increased temperatures for the plant would be speeding up pollen tube growth, viability of fertilized ovules and, ultimately, seed and fruit set (Kjellberg et al., 1982; Erickson and Markhart, 2002).

From an evolutionary perspective, the high water costs of corollas in hot and dry environments can influence flower size. Corolla tube length of long-flowered gilly, *Ipomopsis longiflora*, was affected by both high temperature and water stress (Villarreal and Freeman, 1990). Drought conditions favor selection toward small-flowered plants through female fitness in the alpine wildflower *P. viscosum* (Galen, 2000) and plants producing fewer flowers with shorter corollas in the short-lived perennial *Lobelia siphilitica* (Caruso, 2006). Likewise, non-irrigated plants produced shorted corollas in the long and large-flowered perennial *Datura wrightii* (Elle and Hare, 2002) and smaller flowers with less nectar in the Indian tobacco, *Nicotiana quadrivalvis* (Halpern et al., 2010). In the Mediterranean, a pattern of increasing corolla size with milder conditions was reported both in the drought-tolerant shrub *Rosmarinus officinalis* in southern Spain (Herrera, 2005) and in the daffodil *Narcissus triandrus* along an ecological gradient (Barrett et al., 2004).

Between coflowering sympatric species, differences in flower size contribute to differential pollinator visitation rates and pollen transfer (Aigner, 2005; Vespirini and Pacini, 2010; see also Bosch, 1992 for *Cistus*) and may decrease the reproductive success of smaller-flowered species (reviewed in Jones, 2001). However, the smaller-flowered species could benefit from lower floral costs and reallocate greater resources to reproductive output. By comparing fruit set and seed production of petal-removed flowers and unmanipulated control flowers under controlled pollination conditions between *C. albidus* and *C. ladanifer* in our study population, we detected lower indirect costs of corollas in terms of fruit production in the former species (Teixido and Valladares, 2013). This means that resources directly invested in corollas, such as carbon, nutrients and water, which occur in limited amounts in the natural field sites, translate into indirect costs (Chapin, 1989; Ashman and Schoen, 1997), i.e., negative effects on floral functions other than pollinator attractiveness, such as reproductive output. Therefore, in the studied *Cistus* species, higher floral maintenance costs and derivative indirect costs of corollas in terms of fruit and seed production in the larger-flowered species could counteract the positive effect of greater pollinator attractiveness in this species and, hence, could explain why these two sister species are able to co-flower in sympatry despite their contrasting flower size.

Carbon costs of corollas

Corollas account for the higher respiration rates in the perianth as a whole but these rates are low relative to those of carpels and stamens (Vemmos and Goldwin, 1994; Seymour and Schultze-Motel, 1998; Seymour and Matthews, 2006; Seymour, 2010). For fully-expanded corollas, mean net carbon assimilation rates approached zero in the alpine snow-buttercup *R. adoneus* ($-0.25\,\mu mol\,CO_2\,m^{-2}\,s^{-1}$; Galen et al., 1993). Therefore, mean net carbon exchange rate recorded in corollas of *C. ladanifer* ($-0.91\,\mu mol\,CO_2\,m^{-2}\,s^{-1}$) suggests high respiratory demands in this large-flowered Mediterranean species. Higher floral respiration rates by means of the effect of the alternative oxidase pathway have been reported in thermogenic flowers by modulating the rate of heat production to remain much warmer than ambient temperature (Seymour, 2010). Explanations for these high rates include enhancement of scent production, protection from freezing and a thermal reward to pollinators (Seymour, 2001). Species with thermogenic flowers were the only group where the effects of ambient temperature and flower size on corolla respiration rates were studied. Logically but contrary to our results, Seymour (2001) reported that an increase in ambient temperature decreases the rate of carbon loss in those species, since respiration was coupled to heat production. We did not find evidence of any allometric effect on respiration rates of corolla size. We are aware of some noise introduced by the field protocol: we measured respiration over an air temperature range of $8\,°C$ in comparison to a range of $12\,°C$ when measuring transpiration. However, both the range and mean of temperatures on sunny days for water costs where we recorded a strong allometric relationship between corolla size and transpiration rates were similar to those of carbon costs. In contrast with our results, Seymour (2010) found that heat production (i.e., respiration rate) decreased with flower size to the -0.42 power in 15 species of Araceae. Nevertheless, thermogenesis as measured by Seymour (2001, 2010) is the effect of the alternative oxidase pathway and therefore not directly comparable to our results.

Carbon costs in relation to corolla size were indirectly measured in water use studies of flowers (Galen et al., 1999; Galen, 2000). Galen and colleagues reported that leaf photosynthetic rate at the time of flowering significantly declined with increasing corolla size in *P. viscosum* under dry conditions. Therefore, water use by large corollas influenced leaf stomata closure, constraining carbon gain (see also Lambrecht and Dawson, 2007). This connection between water economy and carbon balance could have evolutionary consequences on flower size in our populations of *C. ladanifer*. As this large-flowered species under hot and dry conditions increases carbon and water losses, by higher respiration and transpiration rates at corollas, respectively, at increasing corolla sizes, reduced leaf photosynthesis in large-flowered plants could translate into additional carbon costs by missed opportunity for carbon gain at leaves and, eventually, giving rise to dysfunctional corollas and lack of photosynthates to set fruits (Galen, 2000; Lambers et al., 2008).

Whole plant water and carbon impacts at flowering

Large flowers of *C. ladanifer* use significant amounts of water and carbon per unit area through their corollas relative to those of leaves (47 and 62% of the water and carbon spent by leaves, respectively and on an organ surface area basis). We did not consider the carbon required for stem respiration, but woody tissues represent only a maximum of 17% of leaf respiration of shrub species in hot and dry environments (Levy and Jarvis, 1998). However, water and carbon use by flowers was actually underestimated because we did not consider construction costs, nectar costs, nor transpiration and respiration by calyx, stamens and carpels, which also

use high amounts of water and energy (Blanke and Lovatt, 1993; Galen et al., 1993, 1999; Patiño and Grace, 2002; De la Barrera and Nobel, 2004b). This whole plant analysis of flower costs is particularly scant in the literature. Some studies had also observed that water loss from flowers can exceed that of leaves in hot and dry environments in *Persea americana* (Whiley et al., 1988; Blanke and Lovatt, 1993) and in *Agave deserti* (Nobel, 1977a). Additionally, Nobel (1977b) reported that flowers of the CAM barrel cactus *Ferocactus acanthodes* use 300 g of water during flowering, about a 2% of the annual transpiration rate of the plant as a whole.

In the Mediterranean, water and carbon shortage are among the main factors limiting plant survival so conservative resource use strategies seem to be adaptative for evergreen woody plants in these ecosystems (Valladares et al., 2000; Thompson, 2005). Large and costly flowers not only contrast with these strategies, but also with resource-cost hypotheses on floral evolution that postulate that reduced corollas are advantageous under stressful conditions (Galen, 1999, 2005). Water and carbon costs of flowering in *C. ladanifer* are, however, consistent with its low efficiency in photosynthetic water use. This drought-avoiding schlerophyllous shrub fully exploits the brief favorable conditions of spring to maximize carbon assimilation at the expense of high rates of leaf transpiration (Valladares et al., 2004). Large corolla size in this species fits well with this water spending strategy of the plant that can be afforded only during a relatively brief spring period.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ppees.2014.02.002.

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