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XXX Cycle

Adaptive strategies of *Cistus* species to Mediterranean stress factors

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Summary

Plant functional diversity is achieved by trade-offs between physiological and morphological traits which are now widely used to define plants' adaptive strategies to cope with environmental changes.

Under Mediterranean conditions, species have to face two main stress factors, namely winter chilling and summer drought. Such factors show a great spatial variability through the Mediterranean Basin and contributed to diversify plant species adaptive strategies. Furthermore, local plant populations within the Mediterranean Basin can be exposed to different selective pressures possibly affecting their adaptive strategies in response to the same environmental cues. Common garden experiments have been proved valuable approaches to disentangle differences in leaf traits among populations of Mediterranean species, particularly in widespread ones.

Among them, the genus *Cistus* (Cistaceae) comprises 21 species which developed with the advent of the Mediterranean climate and contributed to shape the current composition and diversity of the Mediterranean area. They are dominant elements in the Mediterranean shrub communities of European-African-Mediterranean ecosystems.

In this context, the aim of the present thesis was to analyze some aspects of the adaptive strategies of the three widespread *Cistus* spp., namely *C. monspeliensis* L., *C. salvifolius* L. and *C. creticus* subsp. *eriocephalus* (Viv.) Greuter & Burdet) in response to the main limiting factors of the Mediterranean climate. Plants were grown from seeds of different provenances under common garden conditions. In particular, common pattern of response across species growing under the same environmental cues as well as the intraspecific variability in such responses were analyzed.
From an ecological point of view, three main features make these species important to investigate: (i) they have pioneer character, making early growth a key point of their adaptive strategies; (ii) being seasonal dimorphic species they display two leaf types (winter and summer leaves) characterized by a relatively short leaf longevity and relatively low leaf construction costs during a growing season, which seems to be an advantage in habitat under intense stress; (iii) they possess a marked short-term physiological plasticity in response to environmental changes.

However, within these three features, some aspects of *Cistus* spp. adaptive strategies have not yet been clarified. In detail: (i) the early growth of this species in relation to environmental changes has been overlooked so far; (ii) it is not yet clear if these species can actually rely on the formation of different functional leaf 'populations' within a single leaf type in order to face environmental changes; (iii) there is no previous study on the profits that can be achieved by adopting a marked short-term physiological plasticity in response to environmental changes.

To address the broad objective and fill the highlighted gaps of knowledge, different experimental approaches were used making up a total of 3 Chapters (plus General Introduction, General Discussion and Conclusions).

In **Chapter 2,** by fitting non-linear models on seedling height data, it was possible to analyze the relationship between early growth parameters and daily air temperature changes. A parameter that could summarize the temperature dependency of the maximum relative growth rate (RGR_{max}) during the early growth stages for the selected provenances was quantified. Nevertheless, a greater temperature sensitivity of RGR lead to a reduced maximum height reflecting a negative trade-off between the extent of RGR temperature responsiveness and the length of the developmental phases. The findings quantitatively defined provenance dependent strategies by which the selected species cope with daily air temperature variations during early growth.

In **Chapter 3** leaves produced under different climatic conditions through the winter season (i.e. chilling stress) to spring were analyzed in order to highlight the leaf traits involved in determining potential resilience of three *Cistus* spp. to changing environmental conditions and to what extent intraspecific differences could affect such response. The results highlighted a complex mechanism that involves diachronic leaf trait adjustments that are parameter-dependent. The findings also demonstrated the existence of different functional leaf populations in *Cistus* spp. which further extend the current understanding of winter leaves structuring and functioning. Such leaf functional differentiation allows the species to minimize leaf construction costs while maximizing resource acquisition and provide a less costly resilience mechanism in the transition from winter to spring season.

In **Chapter 4**, the short-term physiological response of the considered *Cistus* spp. during an imposed experimental drought and subsequent recovery was analyzed. A positive trade-off between stress and recovery responses during a short-term drought experiment was found, and it was also consistent across species and provenances. The study highlighted a short-term functional strategy which involves a rapid recovery of gas exchange parameters to avoid the progression of photoinhibition. Such response was associated with the lack of leaf shedding. Such short-term strategy is advantageous by allowing the species to hold the more costly summer leaves (compared to winter leaves) in order to maintain relatively high productivity to face prolonged drought events.

Overall, the results demonstrated that *Cistus* spp. are affected by stress imposition but they can recover as soon as favorable conditions are restored. This is achieved through their ability to respond to climate unpredictability by adjusting their photosynthetic organs, morphologically and/or physiologically, in order to enhance their performance during favorable climatic conditions.

Nevertheless, such ability can constrain their response to stochastic changes in thermal amplitudes during a growing season due to climate change thus threatening their competitive ability.

The characterization of plant species responses to the major targets of climate change is important in order to highlight mechanisms that can be further investigated both experimentally and in the field. The reported findings provide a conceptual framework, in terms of experimental designs, traits to be included and mechanisms, that deserve to be applied by including other *Cistus* spp. in the field in order to extend the results at the genus level.

Chapter 1

General Introduction

Plant ecologists have long been interested in quantifying how leaf morphology and physiology vary in response to changing environmental conditions and in understanding their adaptive significance (Wright et al. 2005). The adaptive relationship between plant features and the environment has been observed since XIX sec. (Von Humboldt 1807; Darwin 1859). According to the Darwinian point of view, plant ecologists define plant features in a given environment, as filtered by natural selection, as adaptive traits conferring a higher fitness to a species in that environment (Solbirg 1994). However, plant species display high level of developmental, morphological and physiological plasticity (Gratani 2014). This allows plant species to thrive under different environmental and biotic pressures thus enhancing their survival and persistence in different environments (Sultan 1995, 2000, 2003; Pigliucci 2001; Valladares et al. 2006; Ghalambor et al. 2007; Matesanz and Valladares 2014; Gratani 2014; Puglielli et al. 2015). Phenotypic plasticity is defined as the capacity of a genotype to produce functionally different phenotypes in different environments (Bradshaw 1965; Sultan 1995; Pigliucci 2001; Gratani et al. 2014; Gratani 2014; Matesanz and Valladares 2014). As a result, there is not a single trait attribute for any particular species (Violle et al. 2007; Gratani et al. 2014). Nevertheless, phenotypic plasticity is not related to genetic differences thereby including reversible (acclimatory) responses (Bradshaw 1965; Callahan et al. 1997; Jump and Peñuelas 2005). On the contrary, if genetic differentiation occurs among populations of the same species, such genetic novelties are defined as ecotypes (Gratani et al. 2003; Bonito et al. 2011; Niinemets 2015; Varone et al. 2016).

The plethora of plastic responses in plants led ecologists to group species according to their features (Whittaker 1956, 1975; Walter 1973; Grime 1974, 1977; Ellenberg 1978; Box 1981; Huston and Smith 1987; Smith and Huston 1989; Solbirg 1994). The result of such approach was the definition of Plant Functional Types (PFTs), defined as sets of plants exhibiting similar responses to environmental conditions and having similar effects on the dominant ecosystem processes (Walker 1992; Noble and Gitay 1996). Nevertheless, as claimed by Diaz and Cabido (1997), PFTs approach was challenged by the recognition that functional effect groups (species with a similar effect on one or several ecosystem functions) and functional response groups do not necessarily coincide. However, the legacy of PFTs approach was the definition of standardized lists of the most significant and easily measurable plant and leaf traits which are now at the basis of the so-called trait-based approach (Lavorel et al. 2007). The latter allows to summarize plant functional diversity through a relatively small suite of physiological and morphological traits which contribute to define plant strategies to cope with environmental variations. Many of these traits are considered '*economic traits*' being related to the capacity to acquire, use and conserve resources (Reich et al., 2003; Wright et al. 2004; Reich 2014). In particular, Violle et al. (2007) suggested that traits used at

the level of the individual, from the cell to the whole-organism, have to be defined as morphological, physiological and phenological features which have an impact on performance traits and thus indirectly on fitness.

In the last two decades, a goal of plant ecologists has been therefore to identify the trade-offs among such functional traits in order to elucidate how the coordination among them could drive plants' responses to environmental changes. Particular attention was paid to key traits that have a role in the carbon fixation strategy (Grime et al. 1997; Reich et al. 1997; Westoby et al. 2002; Ackerly 2004). Such approach is now widely used in studies ranging from the level of organism to that of ecosystem (Violle et al. 2007). The trait-based approach provides a quantitative, objective and easy-to-use approach for the determination and comparisons of plant adaptive strategies across species in relation to changes in environmental conditions.

The trait-based investigations have been proved useful for the understanding of trait variations along environmental gradients (Ackerly 2002; Wright et al. 2004; Shipley et al. 2006; Ackerly and Cornwell 2007; Cornwell and Ackerly 2009). However, within-species variations in different environments has often been disregarded (Niinemets 2015). Accordingly, mean values of key plant traits exhibit significant within-species shifts across climatic gradients at both global and local scales (Wright et al. 2005; Moles et al. 2007; Niinemets 2015; Martin et al. 2016) thus reshaping the global patterns of trait covariation. The differentiation of populations varies with climate over both space and time as a consequence of natural selection operating on seedling establishment (Gratani et al. 2003; Jump and Peñuelas 2005; Bonito et al. 2011). This is particularly true within the Mediterranean basin. The Mediterranean climate is characterized by two main features: mild and wet winter and drought in summer. However, Mediterranean climate is strongly affected by the complex topography of the Mediterranean region resulting in a great spatial variability of the Mediterranean climatic features (Giorgi and Lionello 2008).

As a consequence, the Mediterranean flora is a complex mixture of taxa of various biogeographical origins and evolutionary histories (Gratani and Varone 2004). It includes more than 48,000 species of plants, about 20% of the known species, of which approximately 50% are endemic (Cowling et al. 1996; Myers et al. 2000; Blondel et al. 2010, Matesanz and Valladares 2014). The Mediterranean flora derives from *in situ* evolution (indigenous elements) whereas some species colonized the region from adjacent or far-distant regions in various periods in the past (pre-Mediterranean elements) (Blondel and Aronson 1999; Gratani and Varone 2004).

The strong seasonality of the Mediterranean climate contributed to diversify plant species adaptive strategies. In particular, the low temperatures in winter associated to high radiation and the

summer drought are considered the main limiting factors of the Mediterranean climate (Gratani and Varone 2004; Catoni et al. 2012). Accordingly, the highest photosynthetic rates for Mediterranean species occur in spring and autumn, whereas the lowest rates in winter and summer (Flexas et al. 2014).

Nevertheless, the extent to which the photosynthesis rate, as well as other leaf physiological, morphological and growth parameters of a species is affected by winter/summer stress factors may depend on both species-specific adaptations and intraspecific variability (Larcher 2000). The latter is particularly relevant for the most widely distributed Mediterranean plant species (Santiso and Retuerto 2016). An experimental approach to disentangle intraspecific trait variation is provided by common garden experiments which allows an insight into the genetic controls on phenotypes in different populations (Niinemets 2015). Common garden studies have in fact highlighted significant differences in plant and leaf traits among populations of Mediterranean species (Gratani et al. 2003; Gimeno et al. 2009; Bonito et al. 2011; Peguero-Pina et al. 2014, 2017; Varone et al. 2016).

The overlapping of multiple stress factors and their variability result in different functional strategies in Mediterranean species (Gratani and Bombelli 2001; Zunzunegui et al. 2011; Gratani et al. 2017). Among them, drought semi-deciduous *Cistus* spp. are characterized by a seasonal reduction in their transpiring leaf surface area (Bombelli and Gratani 2003). They produce short twigs (brachyblasts) with small leaves at the end of spring-beginning of summer (summer leaves), and long twigs (dolichoblasts) in autumn-winter, with larger and thinner winter leaves (Gratani and Crescente 1997; Werner et al. 1999; Palacio et al. 2006; De Micco and Aronne 2009). Seasonal leaf dimorphism is considered as the main adaptation of these species to the seasonal climatic changes occurring in Mediterranean habitats (Orshan 1972; Christodoulakis et al. 1990; Kyparissis et al. 1997). The genus *Cistus* (Cistaceae) comprises 21 shrub species (50–100 cm in height) most of them (14 species) characterized by a shallow, markedly planar root system expanding where soil moisture availability is depleted throughout summer (Amato and Sarnataro 2001). These species are distributed in the Mediterranean Region, in southern Europe, North Africa and western Asia (Fernández-Mazuecos and Vargas 2010), where they colonize degraded areas (Attaguile et al. 2000). Most *Cistus* species are widespread, but few narrow endemics also exist (Fernández-Mazuecos and Vargas 2010). *Cistus* spp. are indigenous taxa of the Mediterranean flora and are determinants of composition and current diversity of the Mediterranean area (Correia and Ascensão 2017). In particular, they originated from a Mediterranean ancestor which may have generated new lines of evolution by exploiting six pre-existing Mediterranean habitats (Guzmán et al. 2009). Multiple leaf strategies were then successfully essayed in the course of speciation to occupy

particular environments and become part of the dominant elements in the Mediterranean scrub communities (Guzmán et al. 2009).

Among the species of the genus, *C. creticus* subsp. *eriocephalus* is a typical Mediterranean shrub species distributed along the coastal belt of the Central-Eastern Mediterranean (it is absent in France and the Iberian Peninsula), Northern Africa and Western Asia (Abbate Edlmann et al. 1994), extending from sea level to 800 m a.s.l., mainly in arid and warm areas of the maquis and garrigue (Pignatti 1982).

Cistus monspeliensis L. is a lowland shrub species displaying a rather continuous distribution in the Mediterranean Basin, even though it becomes scarcer eastwards. It is also found in the Canary Islands (Fernández-Mazuecos and Vargas 2010). Dense *C. monspeliensis* shrubs are found on poor soils from sea-level to 600–800 m a.s.l., both on calcareous and acidic soils (Fernández-Mazuecos and Vargas 2010), and where holm oak, cork oak and pine woodlands are degraded (Juhren 1966). *C. monspeliensis* is a typical species of the garrigue colonizing wide areas after fire (Quézel 1981, 1985).

Cistus salvifolius L. has a circum-Mediterranean distribution, from Portugal and Morocco to Palestine and the eastern coast of the Black Sea, extending into the south of the Eurosiberian region (Short 1994), over a wide range of habitats (Demoly and Montserrat 1993). *C. salvifolius* does not form dense shrubs, but has a patchy distribution in a wide altitudinal range from sea level to 1800 m a.s.l. (Fernández-Mazuecos and Vargas 2010), often occurring in wooded areas as a component of the understory (Farley and McNeilly 2000).

In such context, the aim of the present thesis was to analyze the adaptive strategies of three widespread *Cistus* spp.: *C. monspeliensis* L., *C. salvifolius* L. and *C. creticus* subsp. *eriocephalus* (Viv.) Greuter & Burdet) in response to the main limiting factors of the Mediterranean climate by using a trait-based approach. Plants were grown from seeds of different provenances under common garden conditions. In particular, the focus was to understand if there were common patterns of response across species growing under the same environmental cues and how the different provenances (intraspecific variability) can affect such responses.

From an ecological and ecophysiological point of view *Cistus* spp. possess some characteristics (Correia and Ascensão 2017) that make them important species to investigate:

(**i**) they have pioneer characteristics which are strictly linked to their regenerative strategy. *Cistus* spp. are obligate seeders that rely on seedling recruitment after a disturbance as a mechanism for population persistence (Troia and Laguna 2015, Correia and Ascensão 2017). Such

strategy implies a set of traits that allow them to deal better with the unpredictable Mediterranean conditions, taking full advantage of periods with higher water availability (Correia and Ascensão 2017). The early growth of this species is therefore a key point of their adaptive strategies. This issue is of particular concern since, by colonizing after disturbance, *Cistus* spp. contribute to soil formation and successional dynamics in Mediterranean ecosystems (de Dato et al. 2013). Despite this, the early growth of this species in relation to environmental changes has been overlooked so far;

(ii) they are characterized by a relatively short leaf longevity with low leaf construction costs as a result of their seasonal-dimorphism, which represents a good mechanism to face the main environmental cues of the Mediterranean climate (i.e. winter low temperature and summer drought). However, it is still not clear if these species can actually rely on the formation of different functional leaf 'populations' within a single leaf type which has been only hypothesized in previous studies (García-Plazaola et al. 2000; Oliveira and Peñuelas 2004);

(iii) *Cistus* spp. possess a large plasticity to environmental changes through morphological and physiological responses. In particular, *Cistus* spp. reduce their transpiring leaf surface area when long-term drought events occur. However, they also show a significant physiological plasticity during short-term drought together with a large recovery capacity.

Moreover, it is important to investigate if such aspects are dependent on the provenance, since this issue has never been tested for *Cistus* spp. under common garden conditions.

The presented results significantly expand the current understanding of *Cistus* spp. adaptive strategies. On the whole, the results highlighted:

(i) provenance-dependent strategies to maximize relative growth rate in response to daily mean air temperature changes during early growth stages (**Chapter 2**);

(ii) the existence of functionally different leaves within the same leaf type (i.e. winter leaves) as a resilience mechanism in the transition from winter to spring season associated to a certain degree of intraspecific variability (**Chapter 3**);

(iii) a positive trade-off between the short-term drought and recovery responses which is involved in avoiding the progression of photoinhibition and possibly the premature leaf shedding during short-term drought events independently of provenance (**Chapter 4**).

In the General Discussion (Chapter 5), the reported findings in each chapter are argued to show a correspondence with the pioneer character of these species and to highlight their opportunistic behavior in tracking favorable environmental conditions through the year. Moreover, the possible constrain that such opportunistic behavior can impose to this species in a global climate change context is also provided (Chapter 6).

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Chapter 2

Temperature responsiveness of seedlings maximum relative growth rate in three Mediterranean *Cistus* species

Brief preface

Early growth is a key process for pioneer, fast growing and opportunistic species that usually colonize open habitats. In general, the ecology of *Cistus* spp. is profoundly linked to open and unpredictable environments and their strategy relies on tracking favorable environmental conditions. In **Chapter 2**, a mechanism by which the selected species maximize relative growth rate in response to daily air temperature changes in a provenance-dependent manner is demonstrated. The proposed strategy quantitatively reflects a differentiation that allows *Cistus* spp. populations to cope with the pressure exerted by changes in temporal and temperature windows for seedlings establishment within the Mediterranean Basin. In particular, such mechanism is related to the necessity of hastening the achievement of maximum growth before the unfavorable periods for growth. However, this behavior carries an inevitable functional cost in terms of seedling height.

Temperature responsiveness of seedlings maximum relative growth rate in three Mediterranean *Cistus* species

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Abstract

Aims

Relative growth rate (RGR) is an indicator of the extent to which a species is using its photosynthates for growth and it is affected by environmental factors, including temperature. Nevertheless, most of plant growth studies have been carried out at a single growth temperature or at different temperature treatments, resulting in the lack of information on the relationship between RGR and changing mean daily air temperature. We analyzed the temporal changes in RGR during early growth stages in three *Cistus* species grown outdoor in a common garden from seeds of different provenances. Moreover, we wanted to define the relationship between daily changes in RGR and mean daily air temperature for the considered provenances. The hypothesis that intra-specific temporal variations in RGR can reflect differences in the behavior to maximize RGR (RGR$_{max}$) in response to temperature was tested.

Methods

Seedlings of *C. salvifolius*, *C. monspeliensis* and *C. creticus* subsp. *eriocephalus* were grown outdoor in the experimental garden of the Sapienza University of Rome under a Mediterranean climate. We analyzed early growth with non-linear growth models and calculated function-derived RGRs as the derivative with respect to time of the parameterized functions used to predict height divided by current height. The relationships between function-derived RGRs and mean daily air temperature were analyzed by linear and non-linear

models, which were ranked according to their standard errors and correlation coefficients. The temperature dependency of RGR$_{max}$ per each provenance was evaluated through the relationship between RGR$_{max}$ and the coefficients of the best regression model obtained.

Important Findings

A parameter that could summarize the temperature dependency of RGR up to RGR$_{max}$ during the early growth stages for the selected provenances was defined. This allowed us to highlight that a greater RGR temperature responsiveness was related to a delay in the time to reach RGR$_{max}$ independently by the species. Nevertheless, a greater temperature sensitivity of RGR lead to a reduced maximum height which reflects a negative trade-off between the length of the developmental phases and the extent of RGR temperature responsiveness. Thus, variations in temperature responsiveness of RGR up to RGR$_{max}$ have a significant role in shaping the early growth for the investigated species. Our findings quantitatively define provenance dependent strategies by which the selected species cope with daily air temperature variations during early growth.

Keywords: early growth stage, non-linear growth models, function-derived relative growth rate, local adaptation

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INTRODUCTION

Growth is an important process in understanding plant response to environmental conditions since it integrates across scales from plant to community dynamics and ecosystem

properties (Paine *et al.* 2012). In particular, the early-life cycle stages of a plant are highly sensitive to environmental variations (Bond and van Wilgen 1996; Cochrane *et al.* 2015).

In their natural environments, plants experience large spatial and temporal variations in temperature (Larcher 2000)

and the optimum temperature for growth may potentially differ among and within species (Villar *et al.* 2005).

A useful indicator of the extent to which a species is using its photosynthates for growth is the relative growth rate (RGR) (Gratani *et al.* 2008), which is known to be affected by environmental factors, including temperature (Lambers *et al.* 1998). In particular, maximum RGR (RGR_{max}) is a key trait explaining the distribution of species along environmental gradients, which qualifies it as an important functional trait (Vile *et al.* 2006). During the last decades, there has been an increasing interest to determine RGR_{max} of plant species but most of these studies have been carried out under controlled conditions (laboratory or greenhouse) (Villar *et al.* 2005). This has the advantage of estimating RGR_{max} and to identify the causes of its variation under standard conditions (Villar *et al.* 2005). Nevertheless, most of these studies have been carried out at a single growth temperature (typically 20–25°C) or at different temperature treatments (e.g. Loveyes *et al.* 2002), resulting in the lack of information on the dynamic relationship between RGR and changing mean daily air temperature. The knowledge of both long- and short-term temperature responsiveness of RGR may be useful considering the forecasted increases of daily, seasonal and annual mean temperatures due to global climate change (Atkin *et al.* 2006; Loveys *et al.* 2002). This is of particular importance for those species distributed in the Mediterranean basin which is one of the most prominent 'Hot-Spots' in future climate change projections (Giorgi 2006).

The genus *Cistus* comprises 21 summer drought semideciduous shrub species with a predominantly Mediterranean distribution (Guzmán *et al.* 2009). They are characterized by drought-avoiding phenology, displaying two different leaf cohorts during a year (i.e. summer and winter leaves) (Aronne and De Micco 2001). Their phenological behavior is considered to be the main adaptive feature to the Mediterranean type of climate (Gratani and Crescente 1997). Moreover, *Cistus*

spp. are pioneer species that show enhanced germination and seedling recruitment after fires (de Dato *et al.* 2013). Thus, *Cistus* species are important components of the Mediterranean ecosystems acting as a source of nutrients to the soil and facilitating vegetation succession after disturbance (de Dato *et al.* 2013; Simões *et al.* 2009). A great effort has been made in investigating the germination capability of *Cistus* spp. (e.g. Delgado *et al.* 2008; Olmez *et al.* 2007a,b; Pela *et al.* 2000; Roy and Sonie 1992; Tavşanoğlu and Çatav 2012) and their phenological and physiological adaptations to the Mediterranean climate (e.g. Aronne and De Micco 2001; Catoni *et al.* 2012; de Dato *et al.* 2013). Nevertheless, to the best of our knowledge, the relationship between RGR and temperature in *Cistus* spp. during early growth stages has never been investigated.

Moreover, since under the Mediterranean climate species ability to grow in short periods, when water availability and temperature are favorable, is a key factor in determining the ability to establish itself (El Aou-Ouad *et al.* 2015), the aims of the present study were: (i) to analyze the temporal (i.e. daily) RGR changes during early growth stages of three *Cistus* species (i.e. *C. salvifolius*, *C. monspeliensis* and *C. creticus* subsp. *eriocephalus*) from different provenances and (ii) to define the relationship between RGR and mean daily air temperature for each of the considered provenance. In particular, our hypothesis was that intra-specific temporal variations in RGR reflected differences in the behavior to maximize RGR in response to temperature.

Plant material, study site and climate

Information on the distribution and habitat requirements for the selected species are shown in Table 1.

Seeds of *C. monspeliensis*, *C. salvifolius* and *C. creticus* subsp. *eriocephalus* (hereafter referred as *CM*, *CS* and *CE*, respectively) from different provenances (*n* = 100 seeds per species and provenance) were obtained from the Sardinian Germplasm Bank (BG-SAR). Seeds storage in BG-SAR follows the

Table 1: habitat requirements, altitudinal range (m a.s.l.) and distribution for the three *Cistus* species studied

Species	Habitat	Altitude	Distribution	References
C. creticus subsp. *eriocephalus* Greuter & Burdet (ex *C. incanus* L.)	Coastal belt, arid and warm areas of maquis and garrigue.	0–800	Central-Eastern Mediterranean (it is absent in the Iberian Peninsula), Northern Africa and Western Asia	Abbate Edlmann *et al.* (1994); Pignatti (1982)
C. salvifolius L.	Silicolous and calcicolous soils. Sandy soils of a wide range of habitats; it is often located within the understory.	0–1800	Circum-Mediterranean distribution. South Europe, extending northward to 47° in West France; it is present from Portugal and Morocco to Palestine and the Eastern coast of the Black sea.	Tutin *et al.* (1980); Guzmán *et al.* (2009); Fernández-Mazuecos and Vargas (2010); Farley and McNeilly (2000)
C. monspeliensis L.	Lowland shrub species found on poor calcareous and acidic soils where olm oak and pine woodlands are degraded. It is a typical species of the garrigue.	0–800	Continuous distribution in the Mediterranean Basin even though it becomes scarcer eastwards. It also occurs in the Canary Islands.	Fernández-Mazuecos and Vargas (2010); Quézel (1985)

protocols reported in Bacchetta *et al.* (2008). In particular, *CM* seeds were collected in S-W Spain (referred as *CMSp*, Loc. Pantano Quebrajano, 37°37′57.7″N; 03°43′43.9″W, Andalucía) and in Sardinia (referred as *CMS*, Loc. Guspini, 39°32′32″N; 8°38′02″E, Medio Campidano). *CS* seeds were collected in S-W Spain (referred as *CSSp*, Loc. Huelva, 37°15′N; 6°57′W, Andalucia) and in Sardinia (referred as *CSS*, Loc. Portixeddu, 39°26′32″N; 8°24′37″E, Carbonia-Iglesias). *CE* seeds were collected in Sardinia (referred as *CES*, Loc. Portixeddu, 39°26′32″N; 8°24′37″E, Carbonia-Iglesias) and on the Italian mainland (referred as *CEF*, Loc. Foce del Garigliano, 41°13′23″N; 13°45′45″E, Caserta). The selected provenances fall in a W-E gradient, thus, hereafter *CSSp*, *CMSp* and *CES* are also referred as *eastward provenances* while *CSS*, *CMS* and *CES* as *westward provenances*.

In February 2015, 50 seeds per species and provenance were treated according to Pela *et al.* (2000) as follows: distilled water was boiled to ~100°C and the heat source removed. Then the seeds were soaked in the hot water for 35 s. Seeds were then placed on wet filter paper discs in Petri dishes and incubated in a germination chamber (type CC7, Amcota, Italy). For the 'light' treatment, the following regime was applied: 12:12 h light–dark cycle at 15/6°C. The selected protocol ensures the maximum germination percentage for *Cistus* spp. (Pela *et al.* 2000). The percentage of germination ranged between 32% and 44% for pooled provenances. Thus, 15 replicates per each provenance were established.

In March 2015, seedlings were transplanted to 10 l pots containing an organic commercial substrate (COMPO BIO, COMPO GmbH, Germany) with the following composition: organic carbon (C) 35%, humic carbon 11%, organic nitrogen (N) 1.4%, carbon on total nitrogen ratio of 25, peat (65%) and pH(H$_2$O) 6.0–7.0. Seedlings were arranged in a completely randomized design (i.e. replications are assigned completely at random to independent experimental subjects) and grown outdoor in the experimental garden of the Sapienza University of Rome (41°54′N, 12°31′E; 41 m a.s.l.). Rome has a Mediterranean type of climate (Fig. 1). The mean minimum air temperature of the coldest month (January)

Figure 1: Bagnouls–Gaussen's diagram (time series 2006–14) for Rome. *P* = total monthly precipitation; *T* = mean monthly temperature.

was 4.9°C, the mean maximum air temperature of the hottest month (August) was 31.0°C (data from the Meteorological Station of Roma Macao, Ufficio Idrografico e Mareografico, Lazio Regional Agency, for the period 2006–14). During the study period (May–August 2015), the mean air temperature (T_a) was 21.7±6°C, and total rainfall was 98.6 mm.

Growth analysis

Plants were monitored every 8 days in the period May–August 2015 (i.e. 88 days). In each sampling day, measurements were carried out on 15 randomly selected plants per provenance. Seedling mortality was also recorded. The following parameters were monitored: plant height (*H*, cm), defined as the major distance from the soil level to the highest point of the plant; leaf length (*Ll*, cm) and width (*Lw*, cm), determined in each sampling occasion on 60 fully expanded young leaves per provenance; total number of leaves produced (*n*) and total number of internodes produced (*n*). The ratio between *Lw* and *Ll* (*Lw*/*Ll*) was used as a leaf shape index (Guzmán *et al.* 2009). Leaf production rate (LPR) was calculated according to Cochrane *et al.* (2015) as the natural log of the difference between the number of leaves at first sampling (May 2015) and the number of leaves at the end of the growth period (August 2015), divided by the number of days between the two time periods. Measurements were carried out until no significant differences in *H* between provenances were observed. Leaf mass area (LMA, mg·cm^{-2}) was determined by the ratio between leaf dry mass and leaf area at the end of the study period on 60 fully expanded leaves per provenance.

Given the asymptotic form of *H* data, asymptotic non-linear models were used to describe *H* variations in function of time, following the methodology reported in Paine *et al.* (2012). In particular, the three-parameter logistic, the four-parameter logistic and the Gompertz functions were tested. The three- and four-parameter logistic models were implemented in R (R Development Core Team 2011) with the *SS*logis and *SS*fpl functions, respectively (Pinheiro and Bates 2000). Gompertz models were implemented with the *SS*gompertz function. The fit of these growth functions allows estimation of: initial height (H_0), growth rate of the function (*r*), higher asymptotic height (*K*) and lower asymptotic height (*L*). *H* data were log-transformed in order to reduce heteroscedasticity. The models were ranked by their value of Akaike Information Criterion (AIC). The model with the lowest AIC value (i.e. ΔAIC = 0) was selected as the best model.

Once the best model was selected, average predictions of *H* were generated following the procedure of Araújo *et al.* (2005). In particular, five random samples of the original *H* data, stratified per sampling day, were generated by using Statistica10 (Statsoft, USA). Five runs were made with the best model selected. In each run, the model was calibrated on the 70% random stratified sample of the original *H* data. The predictive accuracy of the model was evaluated on the remaining 30% of the *H* data by simple linear regression
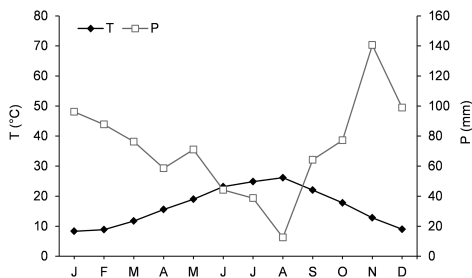
analysis (i.e. Predicted vs. Observed values). The procedure was repeated for each species and provenance.

Models for each provenance were generated by the mean coefficient values (±SD). Averaging predictions is often preferred, since they give the lowest error (Ripley 1996). The obtained models (±SD) were then used to calculate RGR (cm·cm^{-1}·day^{-1}) as the derivative with respect to time of the functions used to predict height divided by current height, according to Paine *et al.* (2012).

Temperature dependency of RGR$_{max}$

The relationship between RGR and mean daily air temperature (T_{mean}, °C) was evaluated through the *Curve finder* function of CurveExpert 1.4 (Hyams Development, TN, USA). This function employs a large number of regression models (both linear and non-linear) and each curve fit is ranked according to its standard error and correlation coefficient. Once the best model was selected, the degree of temperature dependency of the maximum RGR (RGR$_{max}$) per each provenance was evaluated through the relationship between RGR$_{max}$ and the coefficients of the best regression model. T_{mean} data have been supplied from the Meteorological Station of Roma Macao (Ufficio Idrografico e Mareografico, Lazio Regional Agency) for the period May–August 2015. The meteorological station stands 0.84 km far from the experimental site.

RESULTS

Seedling mortality during the course of the experiment was generally low, ranging between 0 and 7% and it was observed during the first 3 weeks of growth analysis, without a clear pattern among the considered provenances.

The three-parameter logistic was best model to describe *H* variations in function of time for all the provenances (Table 2), with mean R^2 ranging between 0.85 (±001) and 0.95 (±0.004) (online supplementary Table S1). The predictive ability of the models (i.e. Predicted vs. Observed values) is shown in online supplementary Table S2. H_{max} did not show significant differences between provenances for both *CM* and *CS* while it was 14% greater ($P < 0.05$) in *CES* than in *CEF* (Table 3, Fig. 2). The number of produced

internodes was positively correlated with *H* ($R = 0.97 \pm 0.01$, $P < 0.0001$) in all the provenances. LPR did not show significant differences between provenances in *CS* and *CE*, while it was significantly higher (by 23%) in *CMS* than in *CMSp* (Table 3). Leaf shape turns from lanceolate-elliptic ($Lw/Ll = 0.35 \pm 0.08$) to linear-lanceolate (0.18 ± 0.06) in *CM*, from ovate (0.58 ± 0.12) to ovate-lanceolate (0.38 ± 0.01) in *CS*, while in *CE* leaf shape was ovate-lanceolate during all the study period with Lw/Ll ranging between 0.31 ± 0.02 and 0.40 ± 0.04. LMA did not show significant difference between provenances in each of the considered species (Table 3).

Table 2: model selection among asymptotic non-linear models used to describe height variations in function of time for: *Cistus monspeliensis* from S-W Spain (*CMSp*) and from Sardinia (*CMS*), *C. salvifolius* from S-W Spain (*CSSp*) and from Sardinia (*CSS*), *C. creticus* subsp. *eriocephalus* from Sardinia (*CES*) and from the Italian mainland (*CEF*)

	Model	ΔAIC
CMSp	Three-parameter logistic	0
	Gompertz	1.1
	Four-parameter logistic	2.38
CMS	Three-parameter logistic	0
	Four-parameter logistic	0.79
	Gompertz	43.06
CSSp	Three-parameter logistic	0
	Four-parameter logistic	1.01
	Gompertz	7.17
CSS	Three-parameter logistic	0
	Four-parameter logistic	1.77
	Gompertz	232.67
CES	Three-parameter logistic	0
	Four-parameter logistic	0.05
	Gompertz	37.68
CEF	Three-parameter logistic	0
	Four-parameter logistic	0.54
	Gompertz	5.37

Models are sorted by ΔAIC.

Table 3: growth and foliar parameters for *Cistus monspeliensis*, *C. salvifolius* and *C. creticus* subsp. *eriocephalus* from different provenances

	H_{max}	RGR$_{max}$	LMA	LPR	*n* internodes
CMSp	3.68 ± 0.04^a	0.052 ± 0.003^a	8.6 ± 1.7^a	0.0175 ± 0.0003^a	23 ± 1^a
CMS	3.64 ± 0.02^a	0.064 ± 0.002^b	8.4 ± 2.2^a	0.0212 ± 0.0001^b	19 ± 1^a
CSSp	3.57 ± 0.02^a	0.048 ± 0.002^a	10.9 ± 3.9^a	0.0186 ± 0.0002^a	17 ± 2^a
CSS	3.49 ± 0.08^a	0.053 ± 0.002^a	8.9 ± 0.7^a	0.0175 ± 0.0001^a	17 ± 1^a
CES	3.59 ± 0.04^a	0.047 ± 0.005^a	10.3 ± 0.7^a	0.0187 ± 0.0001^a	16 ± 1^a
CEF	3.17 ± 0.03^b	0.069 ± 0.004^b	11.2 ± 0.9^a	0.0178 ± 0.0005^a	18 ± 1^a

Mean values ± SD of asymptotic height (H_{max}, log, $n = 5$), function-derived RGR$_{max}$ on a time basis (RGR$_{max}$, cm·cm^{-1} day^{-1}, $n = 5$), LMA (mg·cm^{-2}, $n = 60$), LPR and number of internodes produced are shown. Provenances are labeled as reported in Table 2. Different superscript letters indicate significant intra-specific differences (one-way ANOVA, $P \leq 0.05$).
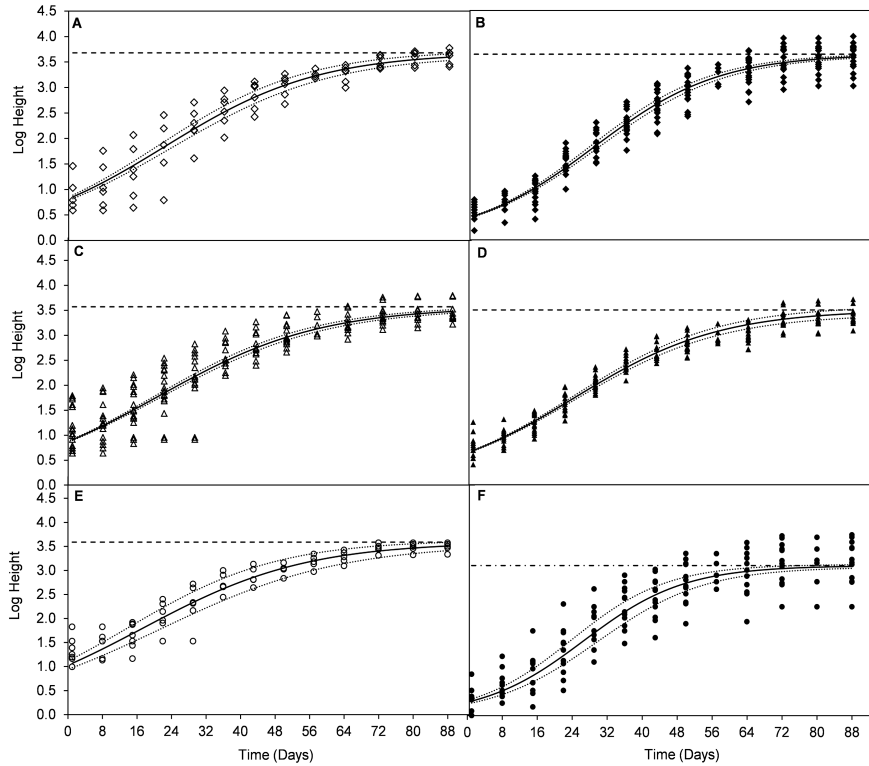
Figure 2: height (log) variations in function of time (days) for: (**A**) *Cistus monspeliensis* from S-W Spain (*CMSp*) and (**B**) from Sardinia (*CMS*), (**C**) *C. salvifolius* from S-W Spain (*CSSp*) and (**D**) from Sardinia (*CSS*), (**E**) *C. creticus* subsp. *eriocephalus* from Sardinia (*CES*) and (**F**) from the Italian mainland (*CEF*). Solid and dashed curves are given by the parameterized functions (±SD, *n* = 5 per provenance) reported in online supplementary Table S1. Horizontal dashed lines show the predicted mean asymptotic height per each provenance (SD is reported in Table 3 and removed for clarity from the figure).

RGR_{max} showed a delay of 6 ± 3 days in individuals from *eastward provenances* (i.e. *CMS*, *CSS* and *CEF*) compared to individuals from *westward provenances* (i.e. *CMSp*, *CSSp*, *CES*) (Fig. 3). RGR_{max} was higher ($P < 0.05$) in *CEF* than in *CES* (0.069 ± 0.006 and 0.047 ± 0.005 cm·cm^{-1}·day^{-1}, respectively) (Table 3) while it was 10% ($P > 0.05$) and 23% ($P < 0.05$) higher in *CSS* and *CMS* than *CSSp* and *CMSp*, respectively.

The relationship between RGR and T_{mean} was described by the Gaussian Model ($R = 0.89 \pm 0.03$) (Table 4, Fig. 4) in the form:

$$RGR = a \times e^{\left(-(b-T_{mean})\right)^2 \big/ \left(2 \times c^2\right)}$$

where *a* is a constant, *b* represents the temperature at which RGR_{max} is reached (T_{max}) (i.e. the horizontal asymptote of the function) and *c* the width of the pick in the curve, which reflects the T_{mean}-dependent relative change of RGR up to

$RGR_{max,}$ and can thus represent a measure of RGR temperature responsiveness (i.e. a lower value of *c* indicates a higher RGR temperature responsiveness) (Table 4). These coefficients were used to analyze the temperature dependency of RGR_{max}. T_{max} and *c* were negatively correlated ($R = -0.84$, $P < 0.0001$). T_{max} was positively correlated with RGR_{max} (slope = 0.01, $R = 0.87$, $P < 0.0001$) while *c* negatively (slope = -0.01, $R = 0.97$, $P < 0.0001$) (Fig. 5). On average, lower T_{max} (by 4%) and higher *c* (by 24%) were observed in individuals from *westward provenances* (i.e. *CMSp*, *CSSp* and *CES*) than individuals from *eastward provenances* (*CMS*, *CSS* and *CEF*) (Table 4, Fig. 5).

DISCUSSION

The growth analysis for the considered *Cistus* species highlights both trait homeostasis and variations between provenances. In particular, leaf parameters (i.e. LPR and LMA)

and the number of internodes produced do not show significant intra-specific differences. The only exception is a significantly higher LPR in *C. monspeliensis* from Sardinia (*CMS*)
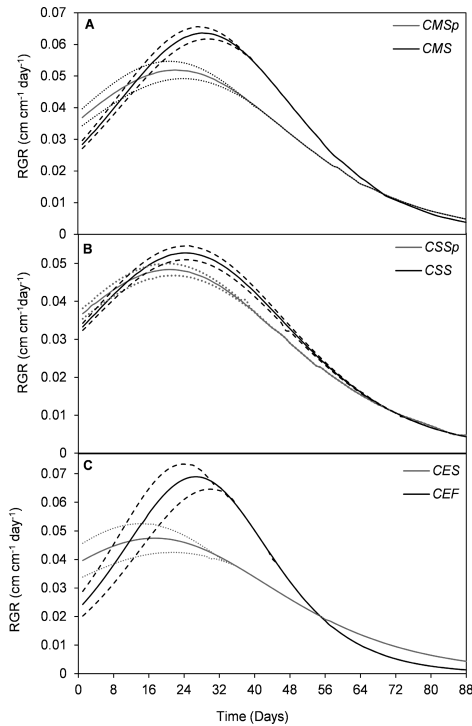

Figure 3: function-derived RGRs on a time basis (RGR, cm·cm^{-1}·day^{-1}) for: (**A**) *Cistus monspeliensis*, (**B**) *C. salvifolius* and (**C**) *C. creticus* subsp. *eriocephalus*. Provenances are labeled as reported in Fig. 2. Solid and dashed curves are given by the derivative with respect to time of the parameterized functions reported in online supplementary Table S1 (±SD, *n* = 5 per provenance) divided by current height.

than from Spain (*CMSp*) which reflects a greater (by 21%, $P > 0.05$) number of internodes produced in *CMS*. Moreover, a very low seedling mortality during the first weeks was observed. Hanley and Fenner (1997) show similar results for other *Cistus* species grown with a different nutrient supply. In fact, seedling mortality in *Cistus* species is known to be related to short stressful events within the first weeks after establishment (Quintana *et al.* 2004). Actually, during our study period, stress events did not occur since the mean monthly temperatures did not exceed the mean temperatures for the period 2006–14. Moreover, plants were regularly watered to prevent water stress in absence of rainfall, thus contributing to reduce seedling mortality.

Differences were observed in H_{max}, which decreased in plants from *eastward provenances* (i.e. *CEF*, *CMS* and *CSS*) in respect to *westward provenances*. This result can be justified by analyzing the different responses of RGR_{max} to T_{mean} at the intra-specific level. Individuals from *eastward provenances* have a higher RGR_{max} (on average by 26%) that is reached at a lower *c* value and at a higher *b* (i.e. higher T_{max}). However, the linear regression RGR_{max} vs. T_{max} shows intra-specific differences in T_{max}. ΔT_{max} is 2.03°C in *CE*, followed by *CM* (ΔT_{max} = 1.23°C) and *CS* (ΔT_{max} = 0.64°C). Since T_{max} and *c* are negatively correlated, the differences in T_{max} result in a reduction of *c* by 32%, 13% and 12% in *CEF*, *CMS* and *CSS*, respectively, compared to their westward counterparts. This highlights a higher temperature responsiveness of RGR in individuals from *eastward provenances*, as well as a narrower temperature window within their RGR can be maximized. Villar *et al.* (2005) argue that a different response to changing environmental conditions, such as temperature, light or soil moisture, have to result in different temporal changes in species RGRs during early growth stages. In our experiment, soil moisture was not limiting and light had a marginal effect in consideration of the high correlation coefficient for the regression RGR-T_{mean} (ranging between 0.84 and 0.91). This suggests that the different T_{mean} responsiveness among the considered provenances drive to the slight delay in reaching RGR_{max} for *eastward provenances*. Similar findings are reported by Skálová *et al.* (2016) which highlight temperature as the

Table 4: values for the parameters for the Gaussian model describing the relationship between function-derived RGR on a time basis (RGR, cm·cm^{-1}·day^{-1}) and daily mean air temperature (T_{mean}, °C)

Equation	$y = a \times e^{\left(-(b-x)^2/(2\times c^2)\right)}$				
	a	*b*	*c*	*R*	*n*
CMSp	0.051 (±0.003)	23.80 (±0.20)	6.18 (±0.06)	0.91 (±0.01)	3
CMS	0.058 (±0.002)	24.97 (±0.15)	5.33 (±0.06)	0.84 (±0.01)	3
CSSp	0.048 (±0.001)	23.5 (±0.10)	6.32 (±0.06)	0.91 (±0.01)	3
CSS	0.050 (±0.002)	23.47 (±0.06)	5.56 (±0.06)	0.91 (±0.02)	3
CES	0.047 (±0.004)	22.87 (±0.50)	6.52 (±0.09)	0.89 (±0.02)	3
CEF	0.062 (±0.005)	24.63 (±0.25)	4.40 (±0.02)	0.86 (±0.04)	3

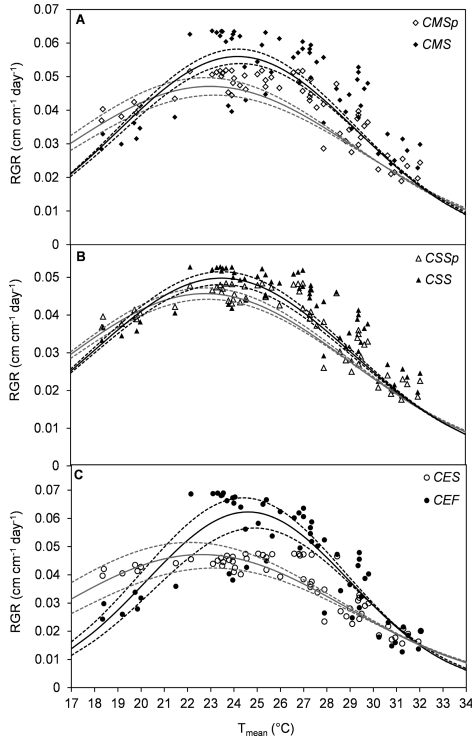Mean values ± SD (*n* = 3 per provenance) of the correlation coefficient (*R*) is also shown.

Figure 4: relationship between daily function-derived RGRs on a time basis (RGR, cm·cm⁻¹·day⁻¹, $n = 88$) and mean daily air temperature (T_{mean}, °C) for: (**A**) *Cistus monspeliensis*, (**B**) *C. salvifolius* and (**C**) *C. creticus* subsp. *eriocephalus*. Provenances are labeled as reported in Fig. 2. Solid and dashed curves are given by the parameterized equations (±SD, $n = 3$ per provenance) reported in Table 4.

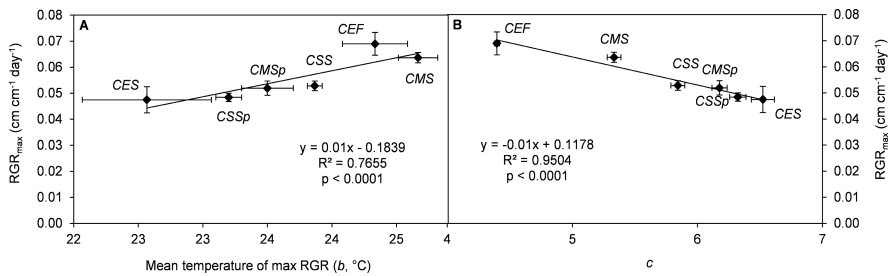main determinant of common ragweed's growth capacity. Moreover, plotting RGR against *H* and comparing the curves in the same range of height (data not shown), RGR is always higher in *eastward* than *westward provenances*, confirming that plants from eastward tend to be fast-growing in a shorter time due to a narrower temperature window (i.e. at higher temperatures). A simple view of the effects of higher temperature on plant size is that the stimulation of growth of existing meristems/organs causes faster development but the duration of phases is shortened (Morison and Lawlor 1999). Thus, the time to supply the assimilated to the growing organs decreases resulting in fewer and smaller organs which determine a smaller size (Morison and Lawlor 1999). This fits the observed trend of the H_{max} decrease associated to the higher RGR_{max} moving eastward, nevertheless, the differences in ΔT_{max} and *c* reflect a lower sensitivity to T_{mean} for *CM* and *CS*, explaining why these species do not show significant intra-specific differences in H_{max}. Moreover, the lower H_{max} in response to a higher RGR temperature responsiveness up to RGR_{max} deserves particular concern considering that during early growth stages, *Cistus* spp. have a low competitive ability for light (Quintana *et al.* 2004). Niinemets (2015) highlights that the mean annual temperature is positively correlated with the longitude ($R = 0.367$, $n = 323$, $P < 0.001$) in the Mediterranean basin, justifying that eastern individuals reach RGR_{max} at lower *c* and at higher T_{max}. Considering that this is associated to a slight delay in reaching RGR_{max}, this confirms that the growth period may be shortened for the eastern plants of the selected species. The multiple regression analysis confirms that RGR_{max} is dependent on the longitude.

Thus, the forecasted increase of air temperature in the Mediterranean basin (Giorgi and Lionello 2008) could alter the highlighted trade-off (i.e. RGR_{max} vs. T_{max}, *c*). In particular, the increase in temperature could result in a higher RGR_{max} in a shorter time resulting in an even smaller size. This can alter the competitive ability of the selected species since plant height

Figure 5: linear regressions analysis between: (**a**) RGR_{max} (cm·cm⁻¹·day⁻¹) and the value of mean daily temperature to reach RGR_{max} (mean daily temperature for RGR_{max}, °C), corresponding to the coefficient *b* reported in Table 4, and (**b**) RGR_{max} (cm·cm⁻¹·day⁻¹) and the relative change of RGR up to RGR_{max}, corresponding to the coefficient *c* reported in Table 4. Provenances are labeled as reported in Fig. 2 (pooled data for all the considered provenances, $n = 18$, $P < 0.0001$).

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is allometrically related to biomass, which in turn is strongly correlated with the fitness (Ackerly *et al.* 2000). Cochrane *et al.* (2015) argue that species developing under Mediterranean conditions can buffer the increase in air temperature by the early seedling emergence in cool winter months. This seems unlikely for *Cistus* species since cold treatments do not increase *Cistus* spp. germination either in greenhouse or field experiments, even if heat pre-treatments are imposed (Nadal *et al.* 2002; Olmez *et al.* 2007a,b). Moreover, high seedling mortality is observed in winter for *Cistus* spp. (Quintana *et al.* 2004), suggesting that seedling establishment and the early growth for the selected species could fall mainly in autumn and spring.

From an ecological point of view, a lower c to reach RGR_{max} when temperature window is narrower represents a mechanism that favors seedling recruitment to a great extent under Mediterranean conditions where the optimum period for plant growth is shortened by both winter chilling temperatures and summer drought (Cochrane *et al.* 2015; El Aou-Ouad *et al.* 2015; Quintana *et al.* 2004). This is particularly true for *Cistus* spp., which develop in fire prone environments where the seedlings establishment window after fire might be rather narrow, and potentially restricted to the first year after disturbance (Quintana *et al.* 2004). In conclusion, our results confirm our working hypothesis as the *Cistus* species investigated show a provenance dependent behavior in maximizing RGR during early growth stages partially relying on the trade-off RGR_{max} vs. T_{max}, c during the favorable period, be it before winter or the onset of summer drought.

CONCLUSIONS

The results of this study reveal intra-specific variability in temperature dependency of RGR_{max} during the early growth stages in three *Cistus* species. Despite species-specific responses are highlighted, individuals from *eastward provenances* show a higher RGR temperature responsiveness up to RGR_{max} than *westward provenances*. This difference is related to a narrower temporal and temperature window in which maximize RGR moving eastward. Nevertheless, a greater temperature sensitivity of RGR up to RGR_{max} leads to a reduced H_{max} reflecting that the length of the developmental phase is affected by the extent of RGR temperature responsiveness. Since our experiment was carried out under not stressful conditions (i.e. not limiting water, nutrients and light availability), we conclude that variations in temperature responsiveness of RGR up to RGR_{max}, as well as its extent, have a significant role in shaping the early growth for the selected species. Moreover, the highlighted variations appear to be genetically fixed and were not dependent from the species.

According to the ecology of *Cistus* spp., our findings quantitatively define provenance related strategies by which the selected species cope with daily air temperature variations during early growth and provide a parameter that summarize the temperature responsiveness of RGR up to RGR_{max} for the three considered species.

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Supplementary material

Table S1

Parametrized equations (three-parameter logistic) used to describe height (H) variations in function of time (t) for *C. monspeliensis* from S-W Sapin (*CMSp*) and from Sardinia (*CMS*), *C. salvifolius* from S-W Spain (*CSSp*) and from Sardinia (*CSS*), *C. creticus* subsp. *eriocephalus* from Sardinia (*CES*) and from the Italian mainland (*CEF*). Coefficients are shown as mean values ± SD of five replicates generated by random samples stratified per sampling day of original H data. Coefficients of determination (R^2) are also shown.

	Three-parameter logistic	R^2	n
CMSp	$H = 3.016[\pm 0.146] / 0.818[\pm 0.032] + 2.866[\pm 0.030]\ e^{-(0.056[\pm 0.002]*t)}$	0.87±0.008	5
CMS	$H = 1.595[\pm 0.04] / 0.438[\pm 0.009] + 3.203[\pm 0.002]\ e^{-(0.069[\pm 0.001]*t)}$	0.95±0.004	5
CSSp	$H = 3.118[\pm 0.061] / 0.873[\pm 0.015] + 2.693[\pm 0.014]\ e^{-(0.054[\pm 0.001]*t)}$	0.86±0.008	5
CSS	$H = 2.275[\pm 0.03] / 0.652[\pm 0.009] + 2.837[\pm 0.099]\ e^{-(0.060[\pm 0.0006]*t))}$	0.95±0.004	5
CES	$H = 3.700[\pm 0.354] / 1.029[\pm 0.087] + 2.564[\pm 0.005]\ e^{-(0.052[\pm 0.004]*t)}$	0.93±0.005	5
CEF	$H = 0.908[\pm 0.122] / 0.286[\pm 0.040] + 2.889[\pm 0.063]\ e^{-(0.087[\pm 0.004]*t)}$	0.85±0.01	5

Table S2

Simple linear regressions between observed and predicted height values for the models reported in Table 2. Coefficients are shown as mean values ± SD (n = 5). Coefficients of determination (R^2) are also shown. Provenances are labelled as reported in Table S1.

	Predicted vs Observed values	R^2	n
CMSp	Pred = 0.05[±0.24] + 0.99[±0.07]*Obs	0.96±0.02	5
CMS	Pred = 0.16[±0.04] + 0.97[±0.02]*Obs	0.95±0.01	5
CSSp	Pred = 0.27[±0.05] + 0.94[±0.01]*Obs	0.88±0.02	5
CSS	Pred = 0.14[±0.05] + 0.96[±0.02]*Obs	0.96±0.01	5
CES	Pred = 0.42[±0.22] + 0.87[±0.08]*Obs	0.94±0.01	5
CEF	Pred = 0.18[±0.14] + 1.01[±0.09]*Obs	0.88±0.03	5

Chapter 3

Provenance effect on carbon assimilation, photochemistry and leaf morphology in Mediterranean *Cistus* species under chilling stress

Brief preface

Following early growth, *Cistus* seedlings establishment relies on their ability to sequentially face the main limiting factors of the Mediterranean climate, winter chilling and summer drought. **Chapter 3** deals with the former and provides an insight on how these species respond to chilling as well as they resume their physiological activity when favorable conditions are restored. The evidence highlights a complex mechanism that involves diachronic leaf trait adjustments that are parameter-dependent. Ultimately, the findings demonstrate the existence of different functional leaf 'populations' in *Cistus* spp. which further extend the current understanding of winter leaves structuring and functioning. Such leaf functional differentiation allows to minimize leaf construction costs and provides a resilience mechanism in the transition from winter to spring season.
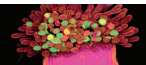
RESEARCH PAPER

Provenance effect on carbon assimilation, photochemistry and leaf morphology in Mediterranean *Cistus* species under chilling stress

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ABSTRACT

- The potential resilience of shrub species to environmental change deserves attention in those areas threatened by climate change, such as the Mediterranean Basin. We asked if leaves produced under different climate conditions through the winter season to spring can highlight the leaf traits involved in determining potential resilience of three *Cistus* spp. to changing environmental conditions and to what extent intraspecific differences affect such a response.

- We analysed carbon assimilation, maximum quantum efficiency of PSII photochemistry (F_v/F_m) and leaf morphological control of the photosynthetic process in leaves formed through the winter season into spring in *C. creticus* subsp. *eriocephalus* (CE), *C. salvifolius* (CS) and *C. monspeliensis* (CM) grown from seed of different provenances under common garden conditions.

- Intraspecific differences were found in F_v/F_m for CE and CS. Carbon assimilation-related parameters were not affected by provenance. Moreover, our analysis highlighted that the functional relationships investigated can follow seasonal changes and revealed patterns originating from species-specific differences in LMA arising during the favourable period.

- *Cistus* spp. have great ability to modify the structure and function of their leaves in the mid-term in order to cope with changing environmental conditions. The F_v/F_m response to chilling reveals that susceptibility to photoinhibition is a trait under selection in *Cistus* species. Concerning carbon assimilation, differing ability to control stomatal opening was highlighted between species. Moreover, seasonal changes of the functional relationships investigated can have predictable consequences on species leaf turnover strategies.

INTRODUCTION

During the winter season, Mediterranean plant species can experience stressful conditions due to a combination of high irradiance and suboptimal growth air temperatures (*i.e.*, 0–10 °C; Niinemets 2016; Varone *et al.* 2015). In fact, winter chilling causes a depression of 1/3 to 2/3 of the annual maximum rate of the photosynthetic activity (Larcher 2000; see Flexas *et al.* 2014 for review), thus representing a selection pressure for Mediterranean species (Grant & Incoll 2005). Nevertheless, in Mediterranean ecosystems with a relatively warm climate plant performance during winter has been poorly studied, perhaps due to summer drought constraints being more conspicuous (Oliveira & Peñuelas 2004). These authors argued that even the mild temperatures of Mediterranean winters might be too low for some plant species that have to cope with wide thermal amplitudes over the year. Although the response to chilling has often been suggested to depend on intraspecific differences (Larcher 2000), this has frequently been ignored (Grant & Incoll 2005). Nevertheless, there is some evidence that morphological and physiological variations exist between

populations of some Mediterranean species, both along large climate gradients (Villar-Salvador *et al.* 1997; Niinemets 2015; González-Zurdo *et al.* 2016) and within smaller geographic areas (Díaz Barradas *et al.* 1999; Grant & Incoll 2005). However, a limited number of common garden experiments (*e.g.*, Lauteri *et al.* 1997; Valladares & Pearcy 1997; Oleksyn *et al.* 2000a,b; Balaguer *et al.* 2001) have been conducted to distinguish if such variations can be referred as 'ecotypic' or 'plastic' sources of variation between species populations within the Mediterranean Basin (Grant & Incoll 2005).

Shrub species are the dominant life form in Mediterranean ecosystems and their potential resilience to environmental change deserves particular attention in those areas threatened by climate change (Grant & Incoll 2005). The genus *Cistus* comprises 21 species that are summer drought semi-deciduous shrubs with a predominantly Mediterranean distribution (Guzman *et al.* 2009). They are representative of the early successional stages in shrublands of the Mediterranean Basin (de Dato *et al.* 2013; Puglielli *et al.* 2017a). Moreover, *Cistus* spp. are found in a wide range of habitats (Nuñez-Olivera *et al.* 1996; Puglielli *et al.* 2017b), hence representing a good test-bed

to address the extent of population differences in the ecophysiological response to limiting factors such as Mediterranean winter chilling. Overall, under moderate chilling, *Cistus* spp. are reported to maintain high rates of photosynthesis, with a large amount of energy dissipated through carbon assimilation (Harley *et al.* 1987; Nuñez-Olivera *et al.* 1996; García-Plazaola *et al.* 2000; Oliveira & Peñuelas 2000, 2002, 2004), very low down-regulation of maximum PSII efficiency (F_v/F_m) and fine environmental modulation of the xanthophyll cycle (García-Plazaola *et al.* 2000). However, permanent photodamage (*i.e.*, F_v/F_m drop) has also been recorded during winter in *Cistus creticus* subsp. *eriocephalus* even if biosynthesis of anthocyanins was increased (Zeliou *et al.* 2009).

Cistus spp. also have two different leaf cohorts within a year (*i.e.*, winter and summer leaves; Aronne & De Micco 2001). Moreover, according to Gratani & Bombelli (1999) and Oliveira & Peñuelas (2004), *Cistus* spp. show an increase in leaf mass to area (LMA) under chilling. A larger LMA implies higher construction cost per unit leaf area (González-Zurdo *et al.* 2016), mainly deriving from its negative relationship with instantaneous carbon assimilation (Reich *et al.* 1997; Niinemets & Sack 2006; González-Zurdo *et al.* 2016; Niinemets 2016). This is paid back in terms of leaf longevity, which leads to important trade-offs between productivity and persistence (Reich 2014; González-Zurdo *et al.* 2016). However, *Cistus* spp. are characterised by a mean leaf longevity of about 6 months (Correia *et al.* 1992; Gratani & Bombelli 1999; Oliveira & Peñuelas 2000), suggesting that leaf morphological changes during winter may not be related to differences in instantaneous carbon assimilation.

In this context, the aim of our study was to investigate *Cistus* spp. intraspecific variability in leaf structural and functional traits in the medium term (months), covering winter and spring periods. Moreover, we wanted to explore the extent to which changes in leaf morphology control the photosynthetic process. To achieve this goal, three *Cistus* spp. from different provenances and grown under common garden conditions were used. According to Niinemets (2016) few data are available on the effects of low temperature on foliage differentiation at the intraspecific level. Thus, analysing leaves from four climatically different experimental periods can provide useful information on the potential resilience of the selected species to changing environmental conditions. In particular, questions include: (i) considering the uncertainty reported in the literature for F_v/F_m response to chilling, can we expect intraspecific differences in this parameter; (ii) given that carbon assimilation is reported as an efficient method to dissipate excess energy under chilling in *Cistus* species, do carbon assimilation-related parameters show intraspecific differences; and (iii) are the expected changes in leaf morphology throughout the winter season related to changes in instantaneous carbon assimilation, since *Cistus* leaves have a short leaf life-span?

MATERIAL AND METHODS

Plant material and study area

For this study, three widespread *Cistus* spp., namely *C. monspeliensis* (CM), *C. salvifolius* (CS) and *C. creticus* subsp. *eriocephalus* (CE), were selected. Seeds from different provenances (n = 100 per species) were obtained from the Sardinian

Germplasm Bank (BG-SAR). CM seeds were collected in south Spain (referred as CMSp, Loc. Pantano Quebrajano, 37°37′57.7″ N, 03°43′43.9″ W, Andalucía) and Sardinia (referred as CMS, Loc. Guspini, 39°32′32″ N, 8°38′02″ E, Medio Campidano). CS seeds were collected in southwest Spain (referred as CSSp, Loc. Huelva, 37°15′41″ N, 6°57′54″ W, Andalucía) and Sardinia (referred as CSS, Loc. Portixeddu, 39°26′32″ N, 8°24′37″ E, Carbonia-Iglesias). CE seeds were collected in Sardinia (referred as CES, Loc. Portixeddu, 39°26′32″ N, 8°24′37″ E, Carbonia-Iglesias) and on the Italian mainland (referred as CEF, Loc. Foce del Garigliano, 41°13′23″ N, 13°45′45″ E, Caserta). CE seeds from the Italian mainland were chosen because the species is not present in Spain. Details on seed germination protocols are reported in Puglielli *et al.* (2017b). The experiment was carried out under natural conditions inside the Botanical Garden of Sapienza University, Rome (41°53′33″ N, 12°27′54″ E, 17 m a.s.l.). For each species and provenance, 2-year-old plants were selected. This plant age is important since the first 2 years after seedling establishment are critical for determining the successful recovery of *Cistus* spp. populations in the natural environment (Quintana *et al.* 2004).

EXPERIMENTAL PROCEDURE

Thirty-six plants were arranged in a completely randomised design (CRD) with six replicates per species and provenance (*i.e.*, replicates assigned completely at random to independent experimental subjects), and watered to prevent water stress in the absence of rainfall, following Puglielli *et al.* (2017b). The stable, medium-term (months) adjustments of leaf gas exchange, chlorophyll fluorescence, stable carbon isotope composition and leaf morphological parameters (Table 1) through winter chilling and spring recovery were investigated. In order to test for differences in acclimation of leaves to changing environmental conditions, we first marked branches (three per plant) and then followed leaf evolution. The leaves were considered fully expanded when they reached a horizontal position (*i.e.*, *ca.* 0°; Oliveira & Peñuelas 2002). Measurements were thus carried out on fully expanded apical leaves (one leaf per each marked branch). The sampling dates defined four experimental periods (EP; Figure S1, Table 2) when the sampled leaves formed. The first period (EPI) lasted from mid-November to mid-December 2015 with a mean air temperature (T_M, °C) of 10.15 °C and global radiation (GR, W·m^{-2}) of 486 W·m^{-2}. The second period (EPII) lasted from mid-December 2015 to the end of January 2016 (T_M = 7.95 °C, GR = 618 W·m^{-2}). The third period (EPIII) lasted from the beginning of February to the beginning of March 2016 (T_M = 10.99 °C, GR = 421 W·m^{-2}). The fourth period (EPIV) lasted from the beginning of March to the beginning of April 2016 (T_M = 12.70 °C, GR = 571 W·m^{-2}) when recovery occurred. Climate data were obtained from the meteorological station in the Botanical Garden of Sapienza University, Rome.

Gas exchange

Gas exchange measurements were performed at midday using an infrared gas analyser in an open system (LCpro+; ADC, Hoddesdon, UK), equipped with a 6.25 cm^2 leaf chamber (PLC, Parkinson leaf chamber). Net photosynthesis per unit

Table 1. List of leaf traits considered in this study. Abbreviations, units, sampling size per provenance (n, plants) and main role of the considered traits in plant functioning are shown.

Trait	Abbreviation	Unit	n	Functional role
Leaf area	LA	m^2	6	Photosynthetic organ size
Fresh weight	FW	g	6	Photosynthetic organ size
Turgid weight	TW	g	6	Photosynthetic organ size
Dry mass	DM	g	6	Photosynthetic organ size
Leaf mass per area	LMA	$g \cdot m^{-2}$	6	Light and carbon acquisition and water use
Leaf dry matter content	LDMC	$mg \cdot g^{-1}$	6	Carbon acquisition and water use
Leaf thickness	LT	µm	6	Light and carbon acquisition
Saturated net photosynthesis per unit leaf area	A_a	µmol (CO_2) $m^{-2} \cdot s^{-1}$	6	Carbon acquisition
Saturated net photosynthesis per unit leaf dry mass	A_m	nmol (CO_2) $g^{-1} \cdot s^{-1}$	6	Carbon acquisition
Stomatal conductance	g_s	mol (H_2O) $m^{-2} \cdot s^{-1}$	6	Carbon acquisition and water use
Leaf transpiration	E	mmol (H_2O) $m^{-2} \cdot s^{-1}$	6	Carbon acquisition and water use
Intrinsic water use efficiency	iWUE	µmol (CO_2) mol $(H_2O)^{-1}$	6	Carbon acquisition and water use
Apparent carboxylation efficiency	C_e		6	Carbon acquisition
Intercellular to ambient $[CO_2]$ ratio	C_i/C_a		6	Carbon acquisition and water use
Isotopic carbon fraction	$\delta^{13}C$	‰	6	Carbon acquisition and water use efficiency
Maximum quantum efficiency of PSII photochemistry	F_v/F_m		6	Light acquisition and use
Ratio between variable and basal fluorescence	F_v/F_0		6	Light acquisition and use

Table 2. Climate characteristics of the experimental periods (EP): mean daily air temperature (T_M, °C), global radiation (GR, $W \cdot m^{-2}$), photosynthetic photon flux density (PPFD, µmol$\cdot m^{-2} \cdot s^{-1}$), sum of precipitation (P, mm) and air relative humidity (RH, %).

	T_M (°C)			GR ($W \cdot m^{-2}$)		PPFD (µmol$\cdot m^{-2} \cdot s^{-1}$)		P (mm)	RH (%)
	Min	Max	Avg	Max	Avg	Max	Avg	Sum	Avg
EPI	5.79	17.25	10.15	997.36	485.86	1257	313	0.6	81.86
EPII	3.75	15.16	7.95	1092.26	617.71	1252	287	3.2	80.34
EPIII	6.21	17.40	10.99	951.53	421.42	2001	469	17	76.71
EPIV	7.27	20.35	12.70	587.92	571.37	2628	749	5.2	69.05

leaf area (A_a), intercellular CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration (E) were determined at ambient CO_2 (C_a) of 400 ppm. Net photosynthesis per unit leaf mass (A_m) was also determined. Leaf temperature was kept at ambient temperature and varied by 1% during measurements in each EP. Relative humidity was between 50–60% during measurements. Photosynthetic photon flux density (PPFD) of 1500 µmol$\cdot m^{-2} \cdot s^{-1}$ was provided by the light source (LCpro+ Lamp unit; ADC). The diode array of the light source contains blue LEDs at 470 nm wavelength and red ones at 660 nm; 10% are blue. Before each measurement, leaves were acclimated to saturating light conditions (*ca.* 15–20 min). Intrinsic water use efficiency (iWUE) and apparent carboxylation efficiency (C_e) were calculated according to Flexas *et al.* (2001). C_i/C_a was also determined. Since the whole leaf was enclosed in the leaf chamber per each provenance and EP, entire leaves were then excised after physiological measurements and used for subsequent morphological and stable carbon isotope analysis.

Chlorophyll fluorescence

Measurements of chlorophyll fluorescence were carried out on the same leaves used for gas exchange measurements, with a portable modulated fluorometer (OS5p; Opti-Sciences, Hudson, NH, USA). Chlorophyll fluorescence measurements were

carried out at midday at saturating PPFD (*i.e.*, 1500 µmol$\cdot m^{-2} \cdot s^{-1}$) ensuring uniform light distribution on the leaf surface while maintaining inclination of the fluorometer pulse source at 45°. Prior to photosynthesis measurements, F_v/F_m was determined on five to six leaves of each plant after 30 min of dark adaptation in leaf clips. F_0 was obtained with a light pulse of ~0.1 µmol$\cdot m^{-2} \cdot s^{-1}$, and F_m with a light-saturating pulse (~8000 µmol$\cdot m^{-2} \cdot s^{-1}$) for 0.8 s. F_v/F_m was calculated as $(F_m - F_0)/F_m$; F_v/F_0 was also determined. These values were then compared to those measured after completion of gas exchange measurements, and no significant differences were observed. The values measured prior to gas exchange measurements were then used in further analyses.

Stable isotope analysis

Measurements of $\delta^{13}C$ were performed with continuous flow IRMS (Conflo IV Universal Interface) using an elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer from Thermo-Fisher Scientific (Bremen, Germany). This system was equipped with ISODAT Thermo Scientific software v 3.0 (Thermo-Fisher Scientific) that was used to acquire and process the signal obtained by IRMS. Wheat flour ($\delta^{13}C = 27.21‰$) provided by IVA Analysentechnik e.K. (Düsseldorf, Germany), cellulose (IAEA-CH3; $\delta^{13}C = 24.72‰$)

provided by the International Atomic Energy Agency (Vienna, Austria) and sorghum flour ($\delta^{13}C = 13.68‰$) provided by Elemental Microanalysis (Okehampton, UK) were used as standards within the weight ranges of 500 ± 30 µg. Samples (range 450–500 µg) were weighed into tin capsules (3.3 mm × 5 mm). The analyses were carried out in batches of ten samples, with standards between each as internal control. The carrier and reference gases were helium in both cases and circulated at 100 and 200 ml·min^{-1} respectively. Temperatures of the elemental analyser were set at 65 °C for the oven, and 650 °C and 1020 °C for oxidation and reduction reactors, respectively. The results of the carbon isotope ratio ($\delta^{13}C$) analyses are reported per mil (‰) on a relative scale and refer to the international standard, V PDB (Vienna Pee Dee Belemnite), according to the following equation: $\delta^{13}C$ (‰) = [($R_{sample/Rreference}$)−1] × 1000, where $\delta^{13}C$ is the ratio of the heavy isotope to the light stable isotope of carbon (*e.g.*, $^{13}C/^{12}C$) in the sample (R_{sample}) and in the standard ($R_{reference}$). Calibration of the control gas (CO_2) was performed using the following reference materials: (ii) IAEA-CH7-polyethylene ($\delta^{13}C = 32.15‰$) and IAEACH6-sucrose ($\delta^{13}C = 10.4‰$) for CO_2 gas cylinder calibration. An internal standard was analysed at regular intervals to control repeatability of the measurements and correct for possible drift in the measurements. The relative SD (n = 10) determined using the corresponding reference gas was 0.05% for $\delta^{13}C$. Each sample was analysed in duplicate with variability <0.2‰.

Leaf morphology

The following morphological parameters were determined: LA, excluding petiole, using an Image Analysis System (Delta-T Devices, Cambridge, UK), FM, TW and DM after drying leaves at 70 °C to constant mass. LMA was determined as the ratio between LA and DM, LDMC was calculated as the ratio between TW and DM (Garnier *et al.* 2001). LT was determined on hand-cut, midpoint leaf cross-sections using light microscopy and an image analysis system (Axiovision AC software), according to Puglielli *et al.* (2015).

Data analysis

A two-way ANOVA was performed on the considered variables in order to evaluate the effect of the main factors (*EP* and *Provenance*) and their interaction on the response variables per each species. Multiple comparisons were analysed with a Tukey test. Standardised major axis regression (SMA; Warton *et al.* 2006) was used to analyse relationships between LMA and LDMC, LMA and LT, LDMC and LT, with *Species* as the main factor. Differences in terms of slopes and intercepts were tested with the Likelihood Ratio and Wald statistic, respectively. Multiple comparisons were performed after adjusting *P*-values with the Sidak correction. All analyses were run with the R library SMATR (Warton *et al.* 2012). This analysis allowed the degree of dependence for the considered variables at the intraspecific level to be defined. The same approach was used for relationships between LMA, LDMC and LT against A_a, A_m and $\delta^{13}C$. Simple linear regression was carried out to test if the considered bivariate relationships held across species (*i.e.*, interspecific level) through each EP. Simple linear and non-linear regressions

were further carried out to analyse relationships between A_a and g_s, C_e, F_v/F_m and F_v/F_0. Non-linear regression used the Curve finder function of CurveExpert 1.4 (Hyams Development, TN, USA) according to Puglielli *et al.* (2017b).

Relationships were considered significant at $P < 0.05$.

RESULTS

No significant differences ($P > 0.05$) between provenances were found, either for the considered gas exchange parameters or for $\delta^{13}C$. For all the considered species A_a significantly decreased in EPII (by 27% on average for the considered species), and increased on average by 81% from EPII to EPIV (Fig. 1A–C). C_e followed the same trend, being significantly related to A_a ($R = 0.95$; Table 3). The relationship between A_a and g_s was described by a hyperbolic function for all the considered species (R from 0.54 to 0.72; Fig. 2). C_i/C_a (Fig. 1D–F) scaled negatively with iWUE (Fig. 1G–I; slope = −0.003; $R^2 = 0.71$; $P = 9.17e^{-16}$). On average, $\delta^{13}C$ significantly increased by 6% and 8% in CS and CM from EPI to EPIV (Table 3). CE showed the same trend of $\delta^{13}C$ increase through the study period, even if the differences were not significant.

The lowest F_v/F_m values were measured in EPII for all the species (0.60 ± 0.01, 0.64 ± 0.08 and 0.58 ± 0.001 in CE, CS and CM, respectively). However, a significant difference was only found in EPII between CE and CS provenances. F_v/F_m increased to 0.73 ± 0.01 (average value for the considered species) in EPIV (Fig. 3A–C). Unlike F_v/F_m, F_v/F_0 showed more significant differences between CE and CS provenances through the EPs (Fig. 4D–F). F_v/F_m and F_v/F_0 were significantly ($P < 0.05$) related to A_a across species ($R^2 = 0.48$ and 0.55, respectively; Fig. 4A and B).

Concerning leaf morphology, LMA increased by 10% (on average for the considered species) from EPI to EPII (Table 4). In EPIV, LMA decreased in CS (1%) and in CE (17%) while increasing in CM (5%), compared to the values measured in EPII. Changes in LMA across species were due to changes in both LT ($R^2 = 0.75$, $P = 2.2e^{-16}$) and LDMC ($R^2 = 0.40$, $P = 6.7e^{-8}$). All the relationships were also significant at the intraspecific level (Table S1). LMA and its components were mostly significantly related to A_m both across and within species (Table S1). The relationships between LMA and LT with A_a were mostly not significant (Table S1). The relationships LDMC *versus* A_a were significant at the intraspecific level. $\delta^{13}C$ significantly scaled at the same rate with LMA and LT for all the considered species, while LDMC was not significantly related to $\delta^{13}C$ (Table S1).

Within each EP, the relationships LMA–LDMC and LMA–LT were weaker in EPII ($R^2 = 0.13$ and 0.41, $P = 0.194$ and 0.01, respectively) compared to the rest of the EPs (mean $R^2 = 0.70$ and 0.80 for the relationships LMA–LDMC and LMA–LT, respectively, $P < 0.05$; Fig. 5). LDMC–LT, which was not significant for pooled data ($R^2 = 0.0276$, $P = 0.852$), changed dramatically within each EP (R^2 between 0.07 and 0.66 and slopes between −0.41 and 0.54). The relationships LDMC–LT ($R^2 = 0.66$, $P = 0.001$) and A_m–LMA ($R^2 = 0.80$, $P = 0.003$) were significant only in EPIV (Fig. 5). The relationships $\delta^{13}C$–LMA and $\delta^{13}C$–LT were only significant in EPI ($R^2 = 0.26$ and 0.28, $P = 0.036$ and 0.044, respectively) and in EPII ($R^2 = 0.36$ and 0.29, $P = 0.023$ and 0.048, respectively). The relationship $\delta^{13}C$–LDMC was not significant within each EP.
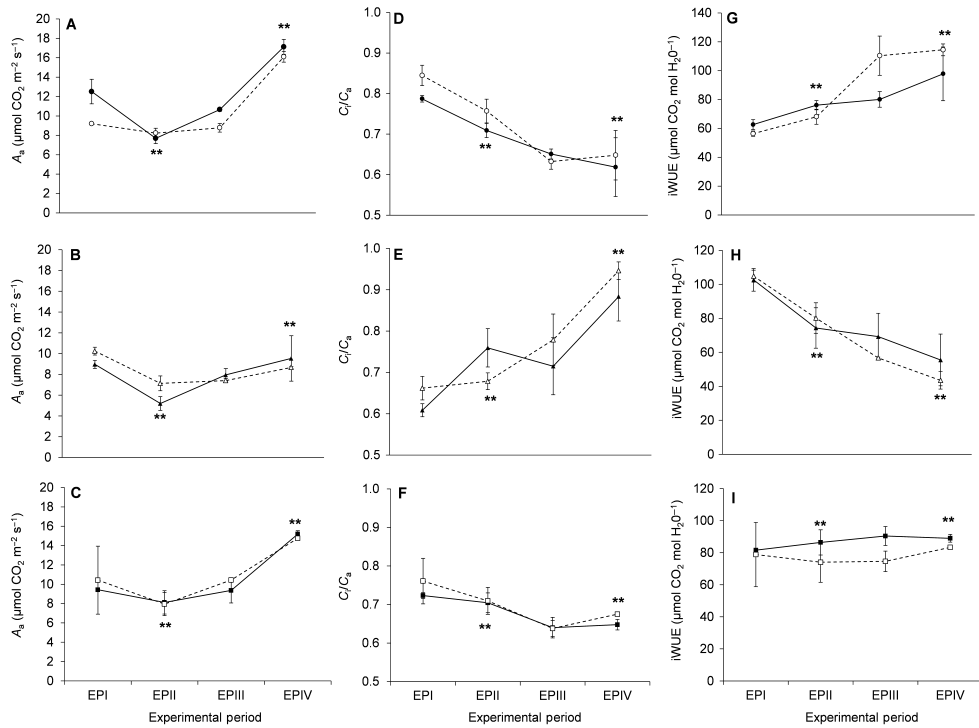
Fig. 1. Trends in saturated net photosynthesis per unit leaf area (A_a) (A–C), intercellular to ambient [CO_2] ratio (C_i/C_a) (D–F) and intrinsic water use efficiency (iWUE) (G–I) for *C. creticus* subsp. *eriocephalus* from the Italian Mainland (CEF, black dots) and from Sardinia (CES, white dots), *C. salvifolius* from southwest Spain (CSSp, black triangles) and from Sardinia (CSS, white triangles), and for *C. monspeliensis* from southwest Spain (CMSp, black squares) and from Sardinia (CMS, white squares) during the four experimental periods (EPs) established. Mean ± SD of six replicates per provenance are shown. No significant differences between provenances are highlighted within each EP; therefore only mean of EPs that significantly differed from all the others are highlighted (two-way ANOVA, **$P \leq 0.05$).

Table 3. Trends in saturated net photosynthesis per unit leaf mass (A_m, nmol·CO_2·g^{-1}·s^{-1}), apparent carboxylation efficiency (C_e) and isotopic carbon fraction ($\delta^{13}C$, ‰) for the considered *Cistus* provenances (labelled as in Fig. 2) during the four experimental periods (EP).

EP	A_m		C_e		$\delta^{13}C$	
	CES	CEF	CES	CEF	CES	CEF
EPI	78.32 ± 11.31a,A	95.39 ± 9.67a,A	0.027 ± 0.001a,A	0.040 ± 0.004 a,A	−32.56 ± 0.01a,A	−31.90 ± 0.22 a,A
EPII	63.95 ± 2.18a, B	75.92 ± 14.94a,B	0.027 ± 0.002a,A	0.035 ± 0.008a,A	−33.28 ± 0.82a,A	−32.26 ± 0.51a,A
EPIII	70.30 ± 0.11a,A	81.81 ± 6.30a,A	0.042 ± 0.001a,A	0.041 ± 0.001a,A	−33.39 ± 0.28a,A	−32.58 ± 0.71a,A
EPIV	145.42 ± 13.52a,C	167.66 ± 3.74a,C	0.062 ± 0.003a,C	0.071 ± 0.010a,C	−32.52 ± 0.61a,B	−32.49 ± 0.42a,A
	CSSp	CSS	CSSp	CSS	CSSp	CSS
EPI	84.03 ± 5.77a,A	89.59 ± 3.51a,A	0.037 ± 0.001a,A	0.039 ± 0.001a,A	−31.38 ± 0.36a,A	−32.78 ± 0.23a,A
EPII	46.83 ± 8.55a, B	54.24 ± 8.98a,B	0.018 ± 0.003a,B	0.027 ± 0.003a,B	−32.60 ± 1.05a,A	−32.05 ± 0.44a,A
EPIII	62.42 ± 5.82a,A	59.36 ± 0.54a,A	0.028 ± 0.001a,A	0.024 ± 0.001a,A	−32.39 ± 0.13a,A	−31.23 ± 0.53a,A
EPIV	77.65 ± 14.79a,C	71.31 ± 5.12a,C	0.032 ± 0.009a,C	0.029 ± 0.001a,C	−30.36 ± 0.53a,B	−30.20 ± 0.26a,B
	CMSp	CMS	CMSp	CMS	CMSp	CMS
EPI	86.73 ± 0.07a,A	99.60 ± 21.01a,A	0.033 ± 0.001a,A	0.036 ± 0.001a,A	−33.60 ± 0.03a,A	−34.72 ± 0.20a,A
EPII	65.97 ± 7.40a, B	67.75 ± 8.89a,B	0.029 ± 0.005a,A	0.029 ± 0.006a,A	−33.44 ± 0.58a,A	−32.56 ± 0.63a,A
EPIII	80.11 ± 9.03a,A	90.39 ± 7.08a,A	0.037 ± 0.007a,A	0.035 ± 0.001a,A	−33.01 ± 0.11a,A	−34.03 ± 0.10a,A
EPIV	93.07 ± 5.45a,C	91.58 ± 7.21a,C	0.058 ± 0.002a,C	0.055 ± 0.001a,C	−31.56 ± 0.79a,B	−30.98 ± 0.20a,B

Mean ± SD of six replicates per provenance are shown. Different lowercase letters indicate differences between provenances within the same EP. Different capital letters indicate within-provenance differences between EPs. Two-way ANOVA, $P \leq 0.05$.
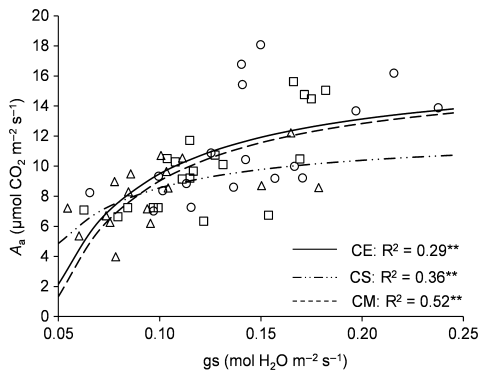
Fig. 2. Regression analysis between saturated net photosynthesis per unit leaf area (A_a) and stomatal conductance (g_s). A hyperbolic function in the form $y = a + b/x$ was fitted to pooled data per species. Mean values per replicate and sampling occasion were used as experimental units. Relationships were considered significant at $P \leq 0.05$. CE, *C. creticus* subsp. *eriocephalus*; CS, *C. salvifolius*; CM, *C. monspeliensis*.

DISCUSSION

Physiological response to winter chilling and recovery

The results showed an overall negative effect of chilling on the physiological activity of the selected species. In particular, as expected, a down-regulation of carbon assimilation and

photochemical parameters was observed. The lowest values of A_a, g_s and F_v/F_m are in the range reported under chilling conditions for *Cistus* species (Munné-Bosch *et al.* 2003; Oliveira & Peñuelas 2004; Catoni *et al.* 2012).

Many authors (Ball *et al.* 1991; Adams & Demmig-Adams 1995; Oliveira & Peñuelas 2004) consider that the reduction in F_v/F_m reflects the action of various protective processes developed by the leaf to use excess absorbed energy not fully utilised in photochemistry. Accordingly, we found that both F_v/F_m and F_v/F_0 scaled with A_a across species and EPs. However, F_v/F_0 was a better predictor of A_a than F_v/F_m. This was due to a partial decoupling of the relationship F_v/F_m–A_a at higher F_v/F_m values. The ratio F_v/F_0 reflects the size and number of active photosynthetic centres in the chloroplasts (Israr *et al.* 2011). Thus, our results strongly suggest that changes in the size and number of active photosynthetic centres in the chloroplasts are involved in decoupling the relationship F_v/F_m, resulting in mostly constant F_v/F_m values (between 0.75 and 0.8; Fig. 4) with varying A_a. This statement is confirmed by a difference in the significant trend of F_v/F_m compared to that of F_v/F_0. Also, the results are in accordance with Grant *et al.* (2014), who highlighted the ability of *C. salvifolius* to vary the structure of light harvesting complexes in the newly formed leaves in response to prevalent environmental conditions. This strategy can contribute to explaining the very low down-regulation of F_v/F_m generally reported for *Cistus* species (see García-Plazaola *et al.* 2000; Oliveira & Peñuelas 2000, 2002, 2004 for further discussion). Nevertheless, significant losses of carbon gain can occur as a result of diurnal photoinhibition, even in the short term (Oliveira & Peñuelas 2004; Grant & Incoll 2005). This is
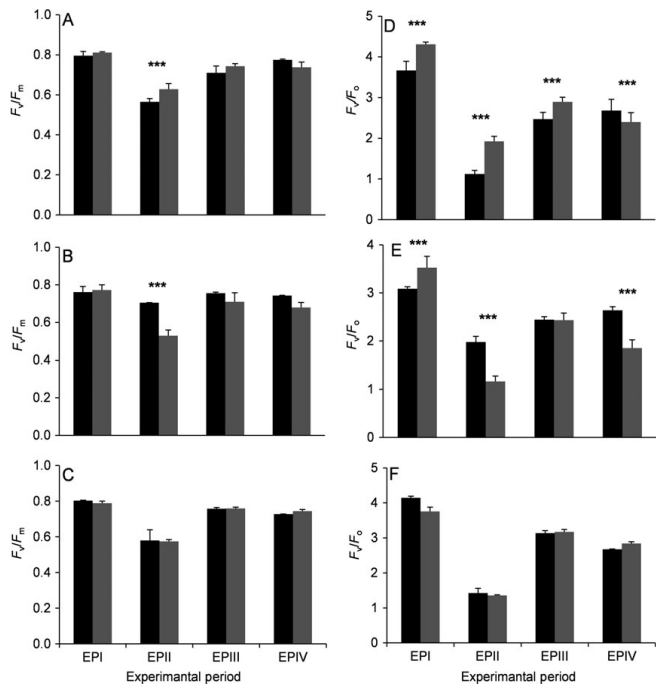

Fig. 3. Trends in maximum quantum efficiency of PSII photochemistry (F_v/F_m) (A–C) and F_v/F_0 (D–F) during the four experimental periods (EP) established for CEF and CES (first empty, black and grey bars, respectively), CSSp and CSS (second empty, black and grey bars, respectively) and CMSp and CMS (third empty, black and grey bars, respectively). Provenances are labelled as in Fig. 2. Mean ± SD of six replicates per provenance are shown. Significant differences between provenances within each EP are shown (Two-way ANOVA, ***$P < 0.001$).
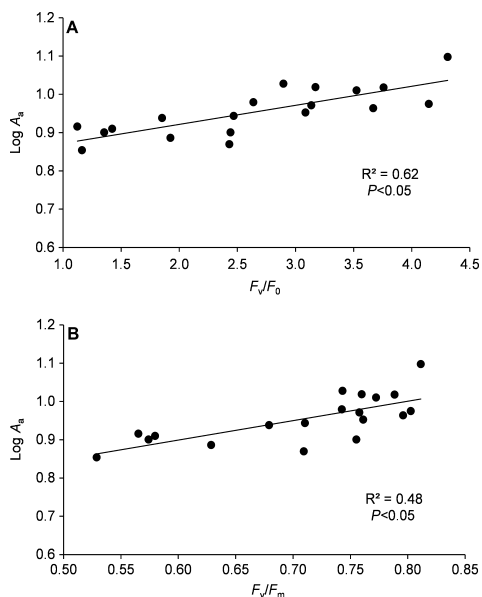
Fig. 4. Regression analysis between (A) log-transformed saturated net photosynthesis per unit leaf area (LogA_a) and stomatal conductance (g_s). A hyperbolic function in the form y = a + b/x was fitted to pooled data per species. Mean values per replicate and sampling occasion were used as experimental units. Relationships were considered significant at $P \leq 0.05$. CE, *C. creticus* subsp. *eriocephalus*; CS, *C. salvifolius*; CM, *C. monspeliensis*.

found within single species (Long *et al.* 1987, 1992; Grant & Incoll 2005). Therefore, selective pressure for sensitivity to photoinhibition is plausible (Long *et al.* 1994). For example, Grant & Incoll (2005) reported variations in susceptibility to photoinhibition in *C. albidus* populations under field conditions. In our study, we found differences in F_v/F_m values only in EPII between CE and CS provenances due to differences in F_v/F_0. This confirms that intraspecific variability in F_v/F_0 can be a target of selection for CE and CS.

We did not find significant intraspecific differences in gas exchange parameters and $\delta^{13}C$. In general, lower temperatures increase the viscosity of water, slowing transport of water from the soil to the evaporative sites in leaves and thus reducing g_s and C_i (Roderick & Berry 2001; Cernusak *et al.* 2013). Under chilling, A_a was much more sensitive to variations in g_s in CE (slope = 24.56) and CM (38.87) compared to CS (3.55). Thus, a decrease in g_s accounts for the C_i/C_a reduction (Cernusak *et al.* 2013) in CE and CM, confirmed by an increase in iWUE and a less negative value of $\delta^{13}C$. In contrast, the observed slight reduction of C_e in EPII for CE and CM can be considered a consequence of both stomatal closure and photoinhibition, rather than a response to chilling. When temperatures become favourable in EPIV, A_a recovery in CE and CM is attained through a disproportionate increase in C_e compared to that of g_s. According to Vitousek *et al.* (1990) a higher carboxylation capacity in relation to g_s decreases C_i/C_a and increases $\delta^{13}C$, as observed in CE and CM. Moreover, slight A_a variations are observed in g_s values between 0.15 and 0.2 mol·H_2O·m^{-2}·s^{-1}. Therefore, a larger A_a than g_s increase in CE and CM in EPIV can explain their higher iWUE.

In contrast, CS showed a C_i/C_a increase in response to chilling, while iWUE decreased. As previously stated, photosynthesis was much less sensitive to g_s variation in CS, possibly indicating the prevalence of non-stomatal limitation of A_a (Flexas *et al.* 2001). However, as the temperature increased,

particularly evident when considering that a tight relationship of $F_v/F_{m-}A_a$ exists at low F_v/F_m values.

At intraspecific level, considerable variation in apparent susceptibility to photoinhibition induced by chilling has been

Table 4. Trends in leaf mass per area (LMA, g·m^{-2}), leaf dry matter content (LDMC, mg·g^{-1}), leaf thickness (LT, µm) for the considered *Cistus* provenances (labelled as in Fig. 2) during the four experimental periods (EP).

EP	LMA		LDMC		LT	
	CEF	CES	CEF	CES	CEF	CES
EPI	131.3 ± 3.8a,A	121.3 ± 17.4a,A	352.6 ± 6.3a,A	340.7 ± 21.9a,A	371.9 ± 7.1a,A	353.1 ± 28.4a,A
EPII	130.4 ± 7.1a,A	128.9 ± 7.3a,A	358.9 ± 6.8a,A	368.1 ± 11.0a,A	363.0 ± 13.6a,A	351.0 ± 25.2a,A
EPIII	131.2 ± 7.9a,A	124.9 ± 6.5a,A	343.4 ± 9.3a,A	343.4 ± 8.8a,B	381.5 ± 12.7a,B	364.4 ± 10.4a,B
EPIV	102.4 ± 6.9a,B	112.7 ± 14.3a,B	276.6 ± 4.4 a,B	309.5 ± 13.2a,B	363.5 ± 23.8a,B	357.9 ± 27.7a,B
	CSSp	CSS	CSSp	CSS	CSSp	CSS
EPI	107.1 ± 4.4a,A	114.9 ± 8.5a,A	300.9 ± 4.5a,A	326.1 ± 6.6b,A	355.8 ± 9.6a,A	352.7 ± 28.9a,A
EPII	113.2 ± 6.5a,A	114.7 ± 11.3a,A	341.7 ± 7.4a,B	352.8 ± 15.3a,B	331.7 ± 18.9a,A	380.3 ± 15.1a,B
EPIII	127.7 ± 2.21a,B	124.7 ± 2.2a,B	338.1 ± 2.6a,B	324.1 ± 0.6a,C	377.5 ± 9.2a,B	384.8 ± 6.2a,B
EPIV	121.4 ± 5.0a,B	122.7 ± 1.0a,B	312.6 ± 3.1a,C	328.9 ± 21.9b,C	388.6 ± 11.7a,B	372.4 ± 5.7a,C
	CMSp	CMS	CMSp	CMS	CMSp	CMS
EPI	108.8 ± 2.1a,A	99.9 ± 12.4a,A	312.2 ± 5.7a,A	318.6 ± 8.5a,A	348.9 ± 0.6a,A	312.1 ± 30.7a,A
EPII	122.2 ± 4.2a,B	116.9 ± 3.7a,B	307.4 ± 7.02a,A	342.5 ± 14.3b,B	398.3 ± 17.8a,B	342.2 ± 11.8b,A
EPIII	116.3 ± 5.7a,B	116.7 ± 8.0a,B	339.9 ± 17.1a,B	328.6 ± 0.6a,C	342.16 ± 6.6a,C	354.0 ± 23.0a,A
EPIV	124.4 ± 13.4a,B	126.3 ± 10.3a,B	340.6 ± 5.2a,B	358.9 ± 10.1b,B	481.4 ± 32.4a,D	454.6 ± 18.3b,B

Mean ± SD of six replicates per provenance are shown. Different lowercase letters indicate differences between provenances within the same EP. Different capital letters indicate within-provenance differences between the EPs. Two-way ANOVA, $P \leq 0.05$.
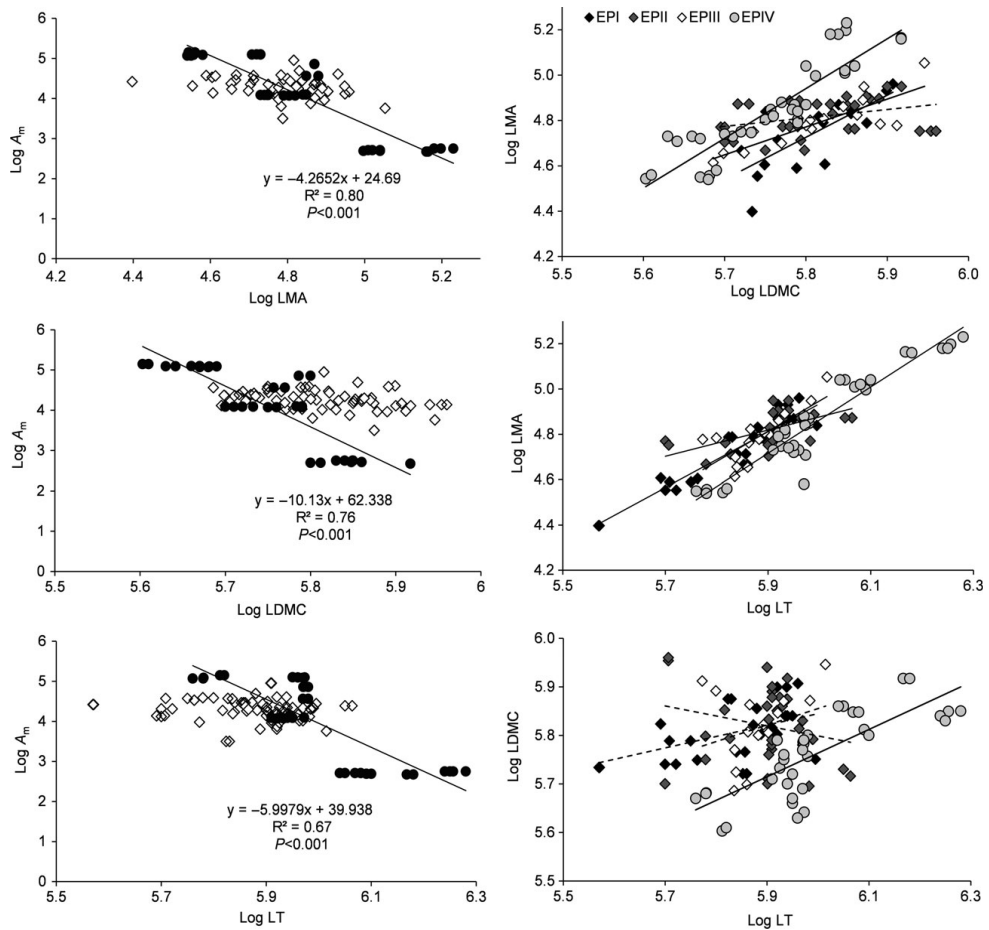
Fig. 5. Regression analysis between leaf mass per area (LMA), leaf dry matter content (LDMC) and leaf thickness (LT) and saturated net photosynthesis per unit leaf dry mass (A_m) (left column) and between LMA and LDMC, LMA and LT and LDMC and LT (right column) within each experimental period (EP). Mean values per replicate and sampling occasion were used as experimental units (n = 36 per EP). Regressions were carried out on pooled data across species per EP. Relationships were considered significant at $P \leq 0.05$. In left column only significant relationships in EPIV are shown (black dots). Dashed lines in right column indicate no significant relationships.

iWUE and C_e remained relatively low. When temperature rises after chilling, a low iWUE is expected to be associated with an increase in C_e, highlighting the predominance of non-stomatal rather than stomatal A_a limitation (Flexas *et al.* 2001). Given the slight C_e increase for CS in EPIV, associated with a relatively low iWUE, we argue that this species is characterised by low stomatal regulation ability through chilling until the spring recovery. This is also highlighted by the less negative values of $\delta^{13}C$ in EPIV. Low ability to regulate stomata has already been reported for *Cistus* spp. (Correia & Catarino 1994). This is particularly true for CS, which is characterised by the most mesic distribution compared to CE and CM, and is the only studied species that can grow in the understorey (see Zunzunegui *et al.* 2016; Puglielli *et al.* 2017a for further discussion).

Morphological control of the photosynthetic process

Leaf morphological parameters varied slightly through the study period, as expected due to the small variation in range of climate variables. Leaf morphological variations in traits were related and scaled with leaf physiological traits, both within and across species. Several studies have reported that LMA can scale with LT (Griffith *et al.* 2016), LDMC (Niinemets 1999; Poorter *et al.* 2009) or both (Niinemets 1999; Wilson *et al.* 1999; Puglielli *et al.* 2015). LT was a better predictor of LMA variations (*i.e.*, higher R^2) than LDMC in our study. Across and within species LMA and its components scaled with photosynthetic capacity, confirming general statements of decreased A_m with increasing LMA

and a general lack of significance in the relationship between LMA and A_a (Wright *et al.* 2004; Niinemets 2015). The highlighted inter- and intraspecific relationships did not hold through each EP to the same extent, and several reports have shown that environmental factors can reshape the well-known trait correlation networks (Lusk *et al.* 2008; Hallik *et al.* 2009; Niinemets 2015, 2016; González-Zurdo *et al.* 2016). The results suggest a possible dependence of the relationship LDMC–LT on mean temperature of EPs. Overall, changes in LMA, LT and LDMC in the considered species reflect some degree of leaf hardening with no effect on the return of carbon fixed under suboptimal conditions. This is confirmed by the lack of significance for the relationships between LMA and its components against A_a and A_m in EPI, EPII and EPIII. Interestingly, we found a positive scaling of $\delta^{13}C$ with LT, and LMA, in EPII. The relative changes of LT in determining LMA variations in EPII may drive internal adjustments in order to reduce the resistance to CO_2 diffusion within the leaves (Puglielli *et al.* 2015). If so, a less-negative foliar $\delta^{13}C$ would be expected in relation to increased resistance to CO_2 diffusion within a leaf (Vitousek *et al.* 1990). However, this was not observed in our study, leading us to argue that the observed changes in LT and LMA in EPII actually aim to reduce resistance to CO_2 diffusion within the mesophyll even if not offsetting the photosynthetic reduction during chilling.

The observed patterns of co-variation between the selected traits were independent of the species or provenance. This is supported by *Cistus* winter leaves having an average leaf life-span of about 6 months (Correia *et al.* 1992; Gratani & Bombelli 1999; Oliveira & Peñuelas 2000), meaning that these species have no need to mirror changes in leaf morphology with a longer payback time for construction costs. Hernandez *et al.* (2011), Saura-Mas & Lloret (2007) and Lloret *et al.* (2016) argued that relatively short-lived species with enhanced establishment after disturbance (*i.e.*, wildfires), such as *Cistus* species, would exhibit increased ability to optimise resource acquisition by minimising construction costs. This was true in our study, since changes in LMA and its components correspond roughly to a constant A_m. Nevertheless, when temperature becomes favourable (*i.e.*, EPIV), then the relationship between LMA (and its components) and A_m was significant and was mainly driven by species-specific differences. In fact, CE and CS decrease LMA, thus maximising the return of carbon fixed per unit leaf biomass, while CM further increases LMA in EPIV. This can result in the newly formed leaves in EPIV being characterised by potential differences in leaf life-span, reducing from CE, CS to CM. Our results suggest that CE and CS would tend to maximise leaf production and may be characterised by higher leaf turnover until the onset of summer drought, while CM would retain different leaf cohorts from the favourable period for growth until the onset of summer drought, which could result in different acclimation capacity to stress factors (Morales *et al.* 2014). This is supported by the literature, reporting that most litterfall occurs during spring in CE and CS (Correia *et al.* 1992; Gratani & Crescente 1997; Fioretto *et al.* 2003; Simões *et al.* 2009), suggesting higher leaf turnover, while this response is delayed in CM until the onset of summer drought (Liberati 2011).

CONCLUSIONS

The results of our study highlight that the investigated *Cistus* species have great ability to modify the structure and function of their leaves in the mid-term in order to cope with changing environmental conditions. This capacity is particularly evident when analysing the photochemical response. The adjustment in size and number of active photosynthetic centres may buffer changes in F_v/F_m. Moreover, this response was related to provenance in two of the three species investigated, revealing that susceptibility to photoinhibition is a trait under selection. Nevertheless, this behaviour was not always sufficient to counteract the negative effects of photoinhibition under chilling. The results otherwise confirm the 'opportunistic' behaviour of these species, which rely on distinct functional leaf populations to reduce the need for antioxidative mechanisms (García-Plazaola *et al.* 2000).

We found a lack of intraspecific differences regarding carbon assimilation. At the interspecific level, we conclude that the difference in response to chilling, as well as to the recovery, is the result of differences in ability to control stomatal opening. In particular, CE and CM had the capacity to adjust stomatal conductance in response to changing environmental conditions, whereas this capacity was lost in CS. Better stomatal control in CE and CM aims to down-regulate the photosynthetic process as a result of limitations imposed by photoinhibition under chilling.

Concerning leaf morphological control of the photosynthetic process, our analysis suggests that the functional relationships analysed can adapt to seasonal changes in *Cistus* spp., with predictable consequences for species leaf turnover strategies and, consequently, ability to withstand drought stress. Moreover, such a response seems to be independent from response to chilling and mainly originates from species-specific differences arising during the favourable period.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Trends in daily mean air temperature (T_M, °C) (black dots). Saily mean minimum and maximum air temperature (grey triangles and squares, respectively). Different shaded areas refer to the four experimental periods (EPs). Black arrows indicate sampling days at end of each EP. Data from the meteorological station of the Botanical Garden of Sapienza University, Rome. More details on climate data are reported in Table 2.

Table S1. Results of standardised major axis (SMA) estimation for bivariate relationships between A_a, A_m and $\delta^{13}C$ and LMA, LDMC and LT for the considered species (see Fig. 2) across experimental periods (n = 144). Note that variables are log-transformed. Slopes and intercepts across and within groups are shown. Different superscript lowercase letters indicate significant differences between slopes and intercept at $P \leq 0.05$. R^2, P-value and results for pooled data across species and experimental periods are also shown. Relationships were considered significant at $P \leq 0.05$.

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Fig. S1. Trend of daily mean air temperature (T_M, °C) (black dots). The daily mean minimum and maximum air temperature are also shown (grey triangles and squares, respectively). Different shaded areas refer to the four experimental periods (EPs) established. Black arrows indicate the sampling days at the end of each EP. Data from the meteorological station of the Botanical Garden of Sapienza University of Rome. More details on climatic data are reported in Table 2.
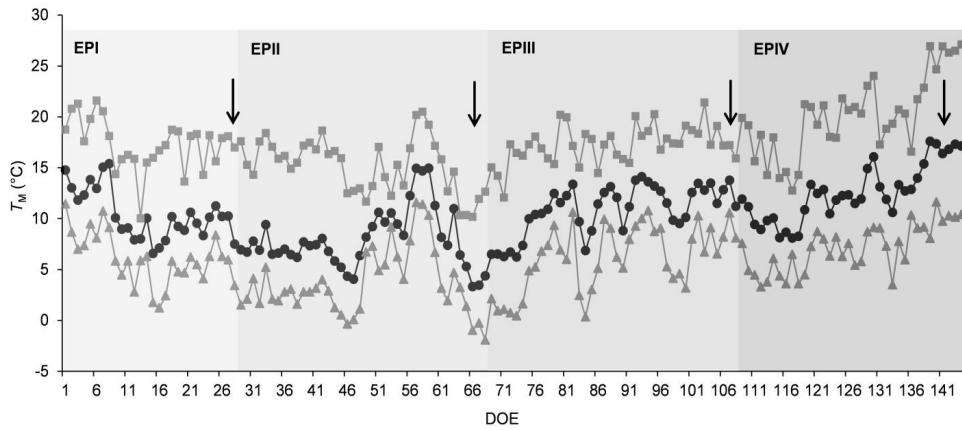
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Species	Response variable	Covariate	Slope	Intercept	R^2	P value	Covariate	Slope	Intercept	R^2	P value	Covariate	Slope	Intercept	R^2	P value
CE	log(A_a)	log(LMA)	1.96	-7.07a	0.12	0.15538	log(LT)	2.61	-12.99a	0.04	0.44307	log(LDMC)	-3.83	24.67a	0.48	1.39E-03
CS				-7.33b	0.01	0.67722			-13.32b	0.04	0.38416			24.25b	0.20	4.71E-02
CM				-7.13a,b	0.51	0.00026			-13.12a,b	0.43	0.00115			24.50a,b	0.21	3.93E-02
Pooled			0.50	-0.17	0.04	0.07731		1.08	-4.11	0.15	0.00235		-0.68	6.21	0.03	2.15E-01
CE	log(A_m)		-3.15	19.59a	0.42	0.00387		-4.18	29.10a	0.68	0.01111		-3.80a	26.59	0.72	7.4E-06
CS				19.23b	0.22	0.03679			28.76a	0.02	0.59113		-4.50a	30.26	0.42	2.1E-03
CM				19.14b	0.56	9.19E-05			28.85a	0.52	2.31E-04		-10.80b	66.61	0.16	6.8E-02
Pooled			-2.22	14.88	0.39	1.20E-07		-2.37	18.20	0.27	2.19E-05		-3.05	21.93	0.20	4.04E-04
CE	$\delta^{13}C$		8.37	-73.08a	0.10	0.26854		10.75	-96.05a	0.37	0.02141		17.20	-133.02a	0.07	0.37288
CS				-71.76b	0.23	3.42E-02			-95.08b	0.48	0.00068			-131.32a,b	0.01	0.69807
CM				-73.30a	0.54	1.45E-04			-96.60a	0.50	0.00034			-132.82c	0.16	0.07200
Pooled			4.50	-54.11	0.22	1.96E-04		6.44	-70.50	0.33	5.19E-06		0.44	-35.06	5.00E-04	0.86750
CE	log(LMA)							1.25	-2.57a	0.53	0.00057		1.37a	-3.15	0.63	8.86E-05
CS									-2.61a	0.66	1.26E-05		1.72a,b	-5.16	0.42	0.00192
CM									-2.60a	0.89	1.60E-10		2.86b	-11.77	0.33	0.00660
Pooled								1.22	-2.30	0.75	< 2.2e-16		1.11	-1.73	0.40	6.70E-08

Chapter 4

Short-term physiological plasticity: trade-off between drought and recovery responses in three Mediterranean *Cistus* species

Brief preface

After winter chilling, *Cistus* spp. maximize their physiological and growth performances to a great extent during spring. Then, drought stress inevitably occurs. These species, being drought semi-deciduous, are known to respond to prolonged drought by shedding most of the leaves thus reducing their transpiring surface. However, the rainfall pattern within the Mediterranean Basin is predicted to change unpredictably in a climate change context. It is therefore likely that Mediterranean species would be subjected to short-term drought events. In such context, leaf shedding may be disadvantageous, in terms of species performance, if favorable conditions are restored in a relatively short time. Moreover, *Cistus* spp. strategy also implies a relatively high recovery capacity. Can therefore stress and recovery capacities equal each other during short-term drought stress and subsequent recovery in *Cistus* spp.? **Chapter 4** answers this question and highlight how this strategy allows these species to retain leaves during short-term drought events.

ORIGINAL RESEARCH

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Short-term physiological plasticity: Trade-off between drought and recovery responses in three Mediterranean *Cistus* species

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Abstract

Short-term physiological plasticity allows plants to thrive in highly variable environments such as the Mediterranean ecosystems. In such context, plants that maximize physiological performance under favorable conditions, such as *Cistus* spp., are generally reported to have a great cost in terms of plasticity (i.e., a high short-term physiological plasticity) due to the severe reduction of physiological performance when stress factors occur. However, *Cistus* spp. also show a noticeable resilience ability in response to stress factors. We hypothesized that in *Cistus* species the short-term physiological response to stress and that to subsequent recovery can show a positive trade-off to offset the costs of the photosynthetic decline under drought. Gas exchange, chlorophyll fluorescence, and water relations were measured in *C. salvifolius*, *C. monspeliensis,* and *C. creticus* subsp. *eriocephalus* during an imposed experimental drought and subsequent recovery. Plants were grown outdoor in common garden conditions from seeds of different provenances. The short-term physiological response to stress and that to recovery were quantified via phenotypic plasticity index (PI_{stress} and $PI_{recovery}$, respectively). A linear regression analysis was used to identify the hypothesized trade-off PI_{stress}−$PI_{recovery}$. Accordingly, we found a positive trade-off between PI_{stress} and $PI_{recovery}$, which was consistent across species and provenances. This result contributes in explaining the profit, more than the cost, of a higher physiological plasticity in response to short-term stress imposition for *Cistus* spp because the costs of a higher PI_{stress} are payed back by an as much higher $PI_{recovery}$. The absence of leaf shedding during short-term drought supports this view. The trade-off well described the relative variations of gas exchange and water relation parameters. Moreover, the results were in accordance with the ecology of this species and provide the first evidence of a consistent trade-off between the short-term physiological responses to drought and recovery phases in Mediterranean species.

KEYWORDS
gas exchange, local adaptations, phenotypic plasticity, resilience

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1 | INTRODUCTION

As the rate of climate change is increasing, it might occur faster than the capacity of plant species to adapt (Ramírez-Valiente, Valladares, Gil, & Aranda, 2009). Phenotypic plasticity rather than genetic diversity is likely to play a crucial role in allowing short-term ecological response of plant species under rapid climate change (Gratani, 2014; Gratani et al., 2014; Puglielli, Crescente, Frattaroli, & Gratani, 2015; Vitasse, et al., 2010). Plasticity may even be adaptive, promoting species establishment and persistence under new climatic conditions (Ghalambor, McKay, Carroll, & Reznick, 2007; Nicotra, et al., 2010). However, some studies show that different environmental conditions can also drive to canalization processes (e.g., Lamy, et al., 2011; Valladares et al., 2002).

Rapid climate change represents a selective force that can drive genetic changes or induce phenotypic plasticity within populations (Jump & Peñuelas, 2005). However, in drought situations, phenotypic plasticity can succeed over evolutionary adaptation (Gimeno, Pías, Lemos-Filho, & Valladares, 2009; Nicotra, Hermes, Jones, & Schlichting, 2007), even blurring ecotypic divergence (Baquedano, Valladares, & Castillo, 2008; Santiso & Retuerto, 2017).

Among the plethora of plastic responses that plant species can exhibit in response to drought, short-term physiological plasticity in traits such as stomatal conductance and photosynthetic rate might be advantageous (Sultan, 2000). Drought represents one of the main limiting factors in Mediterranean ecosystems (Flexas et al., 2014) severely limiting the capacity for CO_2 assimilation (Pastenes, Pimentel, & Lillo, 2005; Puglielli, Redondo-Gómez, Gratani, & Mateos-Naranjo, 2017). However, the plant capacity to face drought stress may depend as much as on the photosynthetic recovery (Varone & Gratani, 2015), which in turn depends on the degree and velocity of photosynthesis decline during water depletion (Chaves, Flexas, & Pinheiro, 2009). Such responses rely on short-term physiological plasticity to contrasting environmental conditions.

Short-term physiological plasticity is particularly important for widely distributed Mediterranean plant species, which represent a good model to investigate whether different provenances respond differently to water stress and recovery due to their short-term physiological plasticity (Santiso & Retuerto, 2017).

Mediterranean *Cistus* spp. show a relatively high physiological plasticity in response to drought (Bongers et al., 2017; Catoni, Gratani, & Varone, 2012; Grant et al., 2014) due to their ability to maximize growth and physiological performance during the favorable periods (Bongers et al., 2017; Puglielli, Spoletini, Fabrini, & Gratani, 2017). Accordingly, a negative trade-off between plant maximum photosynthetic rate in the favorable period and the ability to maintain photosynthesis under adverse conditions has been recently demonstrated (Zhang, Sack, Cao, Wei, & Li, 2017). However, *Cistus* spp. also show a noticeable resilience in their ability to respond to stress factors (see Correia & Ascensão, 2017 for a synthesis; Puglielli, Cuevas Román, et al., 2017).

We therefore hypothesized that the short-term physiological response to stress and that to subsequent recovery, quantified via phenotypic plasticity index (PI, *sensu* Valladares et al., 2000), can show a positive trade-off in order to offset the costs of the photosynthetic decline under adverse conditions. We tested our hypothesis in the three widespread *Cistus* spp. (namely *C. creticus* subsp. *eriocephalus*, *C. monspeliensis*, and *C. salvifolius*) grown from seeds of different provenances under common garden conditions. In particular, we asked the following questions: (1) Can such trade-off be independent of the species and their strategies to cope with drought? (2) Can different provenances of the same species differ in terms of PI thus altering the expected trade-off?

Such evidences are particularly important for *Cistus* spp., considering that they are representative of the early successional stages in the Mediterranean Basin shrublands (Puglielli, Varone, Gratani, & Catoni, 2017). Shrubs' potential resilience to environmental changes deserves particular attention in those areas characterized by pronounced seasonal dynamics and threatened by climate change, such as the Mediterranean ones.

2 | MATERIALS AND METHODS

2.1 | Plant material, study area, and experimental conditions

Seeds of *Cistus monspeliensis* (CM), *C. salvifolius* (CS), and *C. creticus* subsp. *eriocephalus* (CE) from different provenances (*n* = 100 seeds per species) were obtained from Sardinian Germplasm Bank (BG-SAR). In particular, *CM* seeds were collected in South Spain (referred as CMSp, Loc. Pantano Quebrajano, 37°37′57.7″N; 03°43′43.9″W, Andalucía) and in Sardinia (referred as CMS, Loc. Guspini, 39°32′32″N; 8°38′02″E, Medio Campidano). CS seeds were collected in southwest Spain (referred as CSSp, Loc. Huelva, 37°15′41″N; 6°57′54″W, Andalucia) and in Sardinia (referred as CSS, Loc. Portixeddu, 39°26′32″N; 8°24′37″E, Carbonia-Iglesias). CE seeds were collected in Sardinia (referred as CES, Loc. Portixeddu, 39°26′32″N; 8°24′37″E, Carbonia-Iglesias) and on the Italian mainland (referred as CEF, Loc. Foce del Garigliano, 41°13′23″N; 13°45′45″E, Caserta). The choice of CE seeds from the Italian mainland is because the species is not present in Spain. Details on seed germination protocols are reported in Puglielli, Spoletini et al. (2017).

The experiment was carried out under natural conditions inside the Botanical Garden of Sapienza University of Rome (41°53′33″N, 12°27′54″E, 17 m a.s.l.). For each species and provenance, two-year-old plants were selected. This plant age is important since the first 2 years after seedling establishment are critical for determining the successful recovery of *Cistus* spp. population in the natural environment (Quintana, Cruz, Fernández-González, & Moreno, 2004).

Until the onset of the experiment (on 12 July 2016, Day 0), 90 plants (i.e., 3 species × 2 provenances × 15 individuals per provenance) were arranged in a completely randomized design (CRD) and watered regularly to field capacity. We then marked 10 shoots per plant within each provenance which were used for subsequent measurements.

Plant water status, gas exchange, and chlorophyll fluorescence measurements were performed on Day 0 when all the plants were well watered. Thereafter, the water stress was imposed by withholding

water from ten plants from each provenance, and subsequent measurements were carried out every 3 days (namely Day 3, Day 6, Day 9 and Day 12).

The remaining five plants per provenance were kept under daily irrigation. The water stress experiment was stopped when stomatal conductance in stressed plants was below 0.05 mol m^{-2} s^{-1} which is indicative of a severe water stress condition (Medrano, Escalona, Bota, Gulías, & Flexas, 2002). After this threshold, irrigation was restored, and the recovery phase was followed. The recovery was considered to have occurred when stomatal conductance was above 0.20 mol m^{-2} s^{-1}, which is a common value found for *Cistus* well-watered plants in pot experiments (Gallé, Florez-Sarasa, El Aououad, & Flexas, 2011; Puglielli, Redondo-Gómez, et al., 2017). On the whole, the experiment lasted 12 days (the last day is hereafter referred as Day 12).

During the experiment, mean maximum daily air temperature was 34.5 ± 0.7°C, and mean minimum daily air temperature was 22.3 ± 2.1°C. Relative humidity varied between 18 and 56%. Data were obtained from the meteorological station placed in the Botanical Garden of Sapienza University of Rome.

2.2 | Plant water status

Water potential (Ψ_{Pd}, MPa) and relative water content (RWC_{Pd}, %) were measured at predawn on three apical shoots belonging to different plants randomly selected from each provenance per sampling occasion. Ψ_{Pd} was measured by a pressure chamber (SKPM 1400, Sky Instruments, Powys, UK).

RWC_{Pd} was calculated as:

$$[(FW - DW)/(TW - DW)] \times 100,$$

where FW was the apical shoot fresh weight; DW was the dry weight after drying at 80°C until constant weight was reached; and TW was the apical shoot weight after rehydration until saturation in distilled water for 24 hr at 4°C in the darkness.

Being a pot experiment, and considering the below-mentioned destructive measurements necessary to determine shoot gas exchange parameters, Ψ_{Pd}, and RWC_{Pd} were only measured in the three main stages of the imposed stress: Day 0 (first day), Day 6 (maximum stress), and Day 12 (occurred recovery). This allowed us to limit excessive sampling of the available plant material.

2.3 | Shoot gas exchange and chlorophyll fluorescence

Gas exchange measurements were performed using an infrared gas analyzer in an open system (LCpro+, ADC, UK), equipped with a 6.25 cm^2 leaf chamber (PLC, Parkinson Leaf Chamber). Measurements were carried out in the morning (10.00–11.00 a.m.) on all the leaves of three to five apical shoots belonging to different plants and randomly selected from each provenance per sampling occasion. The following parameters were determined: net photosynthesis per unit leaf area (μmol CO$_2$ m^{-2} s^{-1}), stomatal conductance (mol H$_2$O m^{-2} s^{-1}),

transpiration (mmol H$_2$O m^{-2} s^{-1}), and photosynthetic photon flux density (PPFD, μmol photons m^{-2} s^{-1}). Gas exchange parameters were measured at ambient CO$_2$ (of 400 ppm) and leaf temperature (coefficient of variation during measurements of 1%). Relative humidity ranged between 50 and 60% during measurements, and PPFD was always above 1,500.

Measurements of chlorophyll fluorescence were carried out on the same apical shoots of gas exchange measurements, using a portable modulated fluorometer (OS5p, Opti-Sciences, USA).

Chlorophyll fluorescence measurements were carried out at midday at saturating PPFD (i.e., 1,500 μmol photons m^{-2} s^{-1}) ensuring a uniform light distribution on leaf surface while maintaining an inclination of the fluorometer pulse source at 45°. Prior to photosynthesis measurements, maximum quantum efficiency of PSII photochemistry (F_v/F_m) was measured on all the leaves of each apical shoot of each plant after 30 min of dark adaptation with leaf clips. Basal fluorescence (F_0) was obtained with a light pulse of ~0.1 μmol m^{-2} s^{-1}, while maximum fluorescence (F_m) with a light-saturating pulse (~8,000 μmol m^{-2} s^{-1}) for 0.8 s. F_v/F_m was calculated as ($F_m - F_0$)/F_m.

In each sampling occasion, after the completion of gas exchange and chlorophyll fluorescence measurements, the apical shoots were cut, and total leaf area (TLA, m^2) was measured. The final dataset thus included net photosynthesis (A_a, μmol CO$_2$ s^{-1}), stomatal conductance (gs, mol H$_2$O s^{-1}), and transpiration (E, mmol H$_2$O s^{-1}) for the entire apical shoot estimated by multiplying the mean value of each gas exchange parameter per TLA, while F_v/F_m values per each apical shoot were obtained by averaging the F_v/F_m measured for all the leaves of each apical shoot. The choice to express gas exchange parameters normalized by TLA, or as average for the entire shoot in the case of F_v/F_m, was due to the necessity to exclude any difference in leaf number between species and provenances. In order to detect eventual leaf shedding, which is a common response of *Cistus* spp. under drought (Correia & Ascensão, 2017), the total number of leaves per shoot was counted in both stressed and control plants on 10 apical shoots from each provenance and per sampling occasion before physiological measurements.

2.4 | Data analysis

Pearson pairwise correlation coefficients and regression analysis were used to identify bivariate relationships between the considered traits at $p \leq .05$.

The phenotypic plasticity index (PI, Valladares et al., 2000) was calculated for each provenance to quantify the degree of phenotypic plasticity in response to stress (PI_{stress}) and recovery ($PI_{recovery}$) for each of the considered traits. In particular, PI was calculated as: the [(maximum mean–minimum mean)/maximum mean]. For PI_{stress}, the numerator was the difference between the mean value of the trait measured for stressed plants at Day 0 (i.e., first day of the experiment) and at Day 6 (i.e., day of maximum stress), and the denominator was the mean value of the trait measured at Day 0.

$PI_{recovery}$ was calculated by replacing the value of the trait measured at Day 0 with that measured at Day 12 (i.e., maximum recovery). Then,

PI$_{recovery}$ was regressed against PI$_{stress}$ via linear regression analysis (n = 36). In order to prove the predictive ability of the generated linear model, it was cross-validated by randomly assigning the data to five-folds (n = 7–8 per fold). Each fold was removed, in turn, while the remaining data were used to refit the regression model and to predict at the deleted observations. Cross-validation was implemented through the function CV*lm* included in the R package *DAAG* (Maindonald & Braun, 2015).

Differences between provenances in PI$_{stress}$ and PI$_{recovery}$ were tested by one-way ANOVA. Differences were considered significant at p ≤ .05. One-way ANOVA was also used to test for within treatment differences in total leaf number per shoot between sampling days.

Moreover, to test if species shared a common pattern of drought stress response, a principal component analysis (PCA) per species by including all the considered physiological variables grouped by water treatment was carried out. Then, a two-way ANOVA per species on the PC explaining the highest proportion of variance (i.e., PC1) was performed in order to test the effect of the factors provenance (*Pr*), water treatment (*WT*), and their interaction (*Pr*WT*). Multiple comparisons were analyzed by a Tukey test. Such approach was used in order to reduce multiple testing, considering that the use of emerging collective properties (expressed by PC1) as primary variable allows for an equally robust approach (Giuliani, 2017). PCA and two-way ANOVA were performed only on data of the day of maximum stress (Day 6) in order to evaluate if the factor provenance could affect PI estimates.

3 | RESULTS

During stress imposition, Ψ$_{Pd}$ decreased significantly reaching the lowest values −2.23 and −3.4 MPa. At Day 12, Ψ$_{Pd}$ was on average 77% of that measured at Day 0. Pearson pairwise correlation coefficients were all significant highlighting a strong coordination among the physiological variables (mean R = 0.79 ± 0.13). gs, F_v/F_m, and RWC$_{Pd}$ declined in parallel with Ψ$_{Pd}$ and recovered in Day 12 (Figure 1a–c). These relationships also held at the intraspecific level, even if CE and CM showed higher R^2 for the relationships gs–Ψ$_{Pd}$ and F_v/F_m–Ψ$_{Pd}$ compared to CS. The latter, in turn, showed the highest R^2 for the relationship RWC$_{Pd}$–Ψ$_{Pd}$ (Figure 1d–f).

The PCA returned two axes of variation for each species with percentages of explained variance between 78 and 80 for PC1 and between 11 and 14 for PC2. PC1 was in fact correlated (p < .05) with all the physiological variables (Table 1), independently of the species and the provenance, highlighting a reduced performance in stressed plants compared to control ones. On the other hand, PC2 showed an eigenvalue lower than 1, so it was discarded from farther analyses.

The two-way ANOVA showed that the factor *Pr* was significant only in CM (Table 2) even if the significant effect was not due to differences between provenances subjected to the same treatments. This was also true for the significant interaction *Pr*WT* observed for CE

and CS. Overall, as expected, *WT* accounted for most of the variance (Table 2).

No significant differences in mean PI were found between provenances during stress as well as during recovery. Accordingly, PI$_{stress}$ and PI$_{recovery}$ showed roughly the same magnitude (Table 3).

The absence of leaf shedding was observed in both control and stressed plants (Figure 2) with no significant differences throughout the experiment. A highly significant and positive relationship between PI$_{stress}$ and PI$_{recovery}$ across species was found, and the mean square error (MSE) obtained after cross-validation was 0.03 ± 0.01 (Figure 3).

4 | DISCUSSION

A relatively high short-term plasticity was highlighted in the three species independent of the provenance. As such, the investigated provenances show a similar magnitude of decrease in the physiological leaf traits in response to drought as well as a similar increase in reaching the maximum recovery. The similar plastic behavior between provenances may be explained by considering that the physiological adjustments allow species to cope with short-term changes in environmental conditions (Bongers et al., 2017; Gratani et al., 2014; Puglielli, et al., 2015; Puglielli, Redondo-Gómez, et al., 2017 Sultan, 2000). The observed pattern of plasticity was not otherwise necessarily linked to a similar drought response. In fact, the three species show species-specific strategies in drought response. The relationships Ψ$_{Pd}$ versus gs, and RWC$_{Pd}$ highlight that CE and CM are characterized by a greater stomatal control of the photosynthetic process (i.e., higher dependency of gs on Ψ$_{Pd}$ changes, Flexas, Escalona, & Medrano, 1998), whereas CS strategy could rely to a greater extent on osmotic adjustments (i.e., lower dependency of RWC$_{Pd}$ on Ψ$_{Pd}$ changes), according to Catoni, Gratani, Bracco, and Granata (2017).

Phenotypic plasticity has often been assumed to be beneficial under stress conditions (see Gratani, 2014 for a review), even if canalization often occurs at the expense of phenotypic plasticity in stressful environments (Valladares et al., 2002). The convergence in terms of the magnitude of phenotypic plasticity found between these congeneric species as well as between provenances in response to drought seems to integrate both views. The results are also in accordance with the ecology of *Cistus* spp. whose ability to counteract stress factors is constrained by their phenology (Correia & Ascensão, 2017). In fact, *Cistus* spp. are relatively short-lived species that tend to optimize resource acquisition by minimizing construction costs (Hernandez, Pausas, & Vilagrosa, 2011; Lloret, et al., 2016; Puglielli, Cuevas Román, et al., 2017; Saura-Mas & Lloret, 2007). As a consequence, they would rely on physiological plasticity more than morphological in order to counteract short-term stressful events. This statement is supported by the greater physiological than morphological plasticity found by Zunzunegui et al. (2009) for the semideciduous *Halimium halimifolium* in response to stress factors. A widely recognized characteristic of *Cistus* spp. is also their resilience ability (Correia & Ascensão, 2017; Gallé et al., 2011; Puglielli, Cuevas Román, et al., 2017) as they can maximize growth (Correia & Ascensão, 2017; Puglielli, Spoletini, et al., 2017) and improve their performance as soon as the favorable conditions are restored
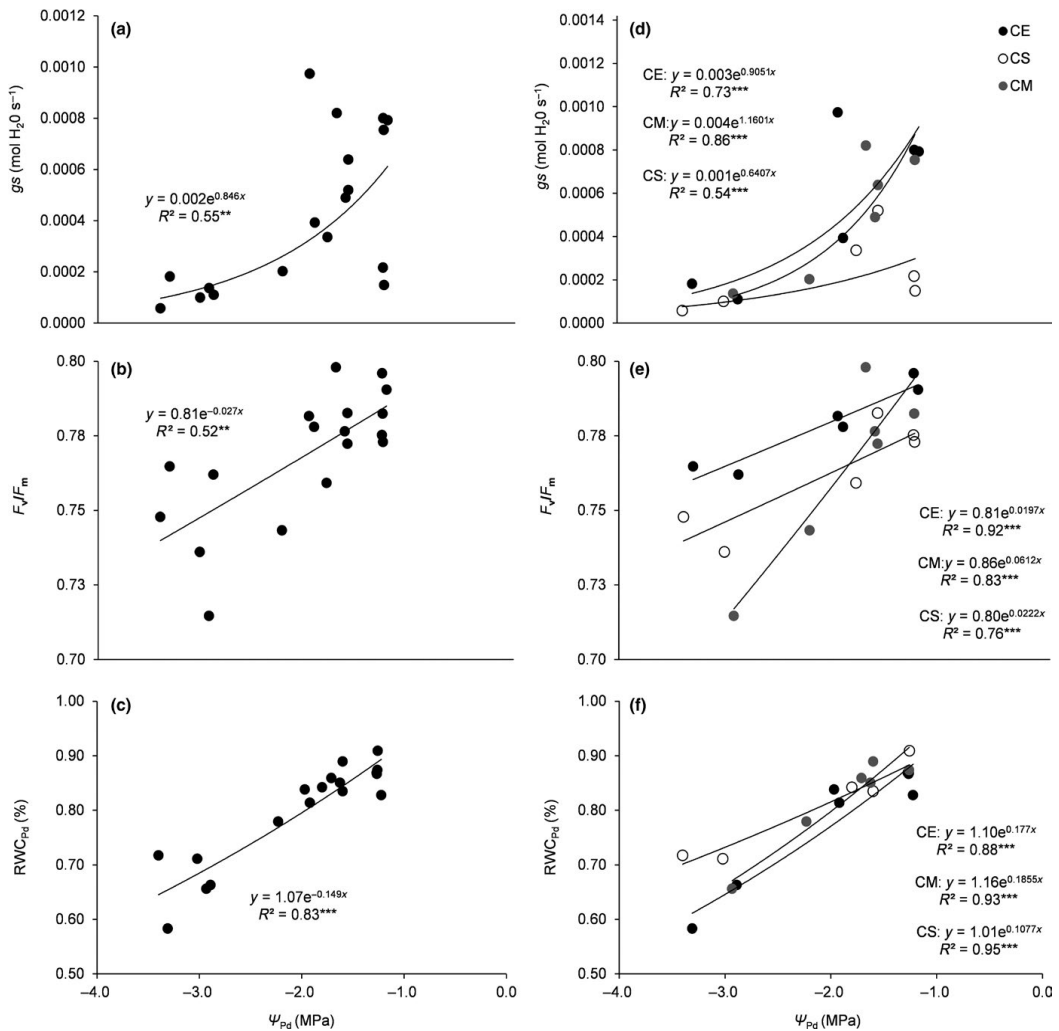
FIGURE 1 Relationship between predawn water potential (Ψ_{Pd}) and (a) stomatal conductance (gs), (b) maximum quantum efficiency of PSII photochemistry (F_v/F_m), and (c) predawn relative water content (RWC$_{Pd}$) across species and days of experiment (n = 3 stressed plants per provenance). The panels d, e, and f display the same relationships within each species. The fitted equations as well as their R^2 are shown. ** and *** indicate p < .01 and .0001, respectively. CE, *Cistus creticus* subs. *eriocephalus*; CS, *C. salvifolius*, and CM, *C. monspeliensis*

(Bongers et al., 2017; Puglielli, Redondo-Gómez, et al., 2017; Puglielli, Cuevas Román, et al., 2017). A high degree of phenotypic plasticity has often been associated with strong performance under favorable conditions (Lortie & Aarssen, 1996; Valladares, Gianoli, & Gómez, 2007). However, the latter was recently related to a lower survival under drought for *C. salvifolius* (Bongers et al., 2017). This evidence suggests that there is an intrinsic cost deriving from the performance maximization during a favorable period and the ability of the species to face, or even survive, drought. Moreover, this is also in accordance with the results of the meta-analysis carried out by Zhang et al. (2017). Our results partly contradict these evidences in terms of physiological plasticity

patterns in the short-term. We found a strong positive relationship between PI$_{stress}$ and PI$_{recovery}$ with most of the points falling in the top right part or tightly around the 1:1 line in Figure 3, underlining that a trade-off exists between the plastic response to stress and that to recovery. The only exception is represented by the combination PI$_{stress}$–PI$_{recovery}$ found for gas exchange parameters in CS, which crossed the 1:1 line. We argue that such a response is related to the lower degree of stomatal control found for CS (i.e., a lower dependency of gs on Ψ_{Pd} changes), which favors a high degree of recovery for gas exchange parameters when favorable conditions are restored. In fact, a lower stomatal control of the photosynthetic process in CS, compared to CE and CM, was

TABLE 1 Factor loadings for principal component analysis (PCA) carried out on the considered physiological traits for *Cistus creticus* subs. *eriocephalus* (CE), *C. salvifolius* (CS), and *C. monspeliensis* (CM)

	CE		CS		CM	
	PC1	PC2	PC1	PC2	PC1	PC2
% Variance	80	14	78	11	79	13
A_a	**0.98**	0.16	**0.97**	0.13	**0.98**	−0.11
gs	**0.94**	0.25	**0.93**	0.34	**0.98**	−0.11
E	**0.98**	0.15	**0.91**	0.38	**0.95**	−0.09
F_v/F_m	**0.80**	0.41	**0.74**	−0.57	0.57	**0.80**
Ψ_{Pd}	**−0.87**	0.46	**−0.89**	0.33	**−0.87**	0.34
RWC_{Pd}	**0.80**	**−0.60**	**0.87**	−0.07	**0.90**	0.17

A_a, net photosynthesis per unit leaf area; gs, stomatal conductance; E, transpiration; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Ψ_{Pd}, predawn water potential; and RWC_{Pd}, predawn relative water content.

The percentage of variance explained by each PC is also shown.

Bold values indicate a significant correlation between the variable and PCs.

TABLE 2 Results of the two-way ANOVA (*F* and *p* values) for the first principal component (PC1) extracted per each species (see Table 1)

Species	Response variable	Factors	F value	p Value
CE	PC1	Pr	1.58	.232
		WT	76.17	**1.50E-06**
		Pr*WT	9.04	**.011**
CS	PC1	Pr	1.78	.196
		WT	205.28	**1.20E-12**
		Pr*WT	6.79	**.016**
CM	PC1	Pr	1.58	.232
		WT	76.17	**1.50E-06**
		Pr*WT	9.04	**.011**

The main effects of provenance (*Pr*), water treatment (*WT*), and the first-order interaction (*Pr*WT*) are shown. Bold values indicate *p* value <.05. Species are labeled as in Table 1.

TABLE 3 Phenotypic plasticity index measured in response to stress (PI_{stress}) and recovery ($PI_{recovery}$) measured per each considered variable for *Cistus monspeliensis from* southwest Spain (CMSp) and from Sardinia (CMS), *C. salvifolius* from southwest Spain (CSSp) and from Sardinia (CSS), and *C. creticus* subsp. *eriocephalus* from Sardinia (CES) and from the Italian mainland (CEF)

	PI_{stress}	$PI_{recovery}$	PI_{stress}	$PI_{recovery}$
	CES		CEF	
A_a	0.75	0.60	0.91	0.82
gs	0.77	0.54	0.86	0.89
E	0.73	0.58	0.90	0.91
F_v/F_m	0.08	0.02	0.07	0.03
Ψ_{Pd}	0.62	0.42	0.58	0.32
RWC_{Pd}	0.33	0.28	0.20	0.21
Mean	0.55	0.41	0.59	0.53
	CSSp		CSS	
A_a	0.71	0.72	0.63	0.80
gs	0.54	0.70	0.61	0.89
E	0.48	0.63	0.32	0.83
F_v/F_m	0.45	0.03	0.45	0.04
Ψ_{Pd}	0.58	0.40	0.63	0.53
RWC_{Pd}	0.18	0.16	0.21	0.14
Mean	0.49	0.44	0.48	0.54
	CMSp		CMS	
A_a	0.71	0.71	0.69	0.72
gs	0.73	0.75	0.72	0.79
E	0.60	0.66	0.78	0.74
F_v/F_m	0.27	0.07	0.27	0.07
Ψ_{Pd}	0.43	0.23	0.45	0.45
RWC_{Pd}	0.11	0.09	0.23	0.26
Mean	0.48	0.42	0.52	0.51

A_a, net photosynthesis per unit leaf area; gs, stomatal conductance; E, transpiration; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Ψ_{Pd}, predawn water potential; and RWC_{Pd}, predawn relative water content. Mean values are also shown.

also found to allow recovery during the transition between winter (i.e., chilling stress) and spring seasons (Puglielli, Cuevas Román, et al., 2017) and it was also reported by Correia and Catarino (1994) in response to drought. We can affirm that CS falls in such trade-off.

Our results support the conclusion that the highlighted trade-off can justify the costs of the greater plasticity in response to stress generally found for *Cistus* spp. (Bongers et al., 2017; Catoni et al., 2012; Grant et al., 2014). Also, the absence of leaf shedding during stress suggests that the observed trade-off may be involved in delaying leaf shedding during short-term stress periods, thus providing a profit for these species. These findings fit the ecological behavior of such species and call for further research on the physiological mechanisms that allow such responses. We argue that one of the mechanisms behind this trade-off is represented by leaf paraheliotropism, a key aspect of the adaptive strategies of *Cistus* spp. (Correia & Ascensão, 2017; Flexas et al., 2014; Gratani & Bombelli, 1999; Oliveira & Peñuelas, 2004; Werner, Correia, & Beyschlag, 1999, 2002), which are known to favor gas exchange
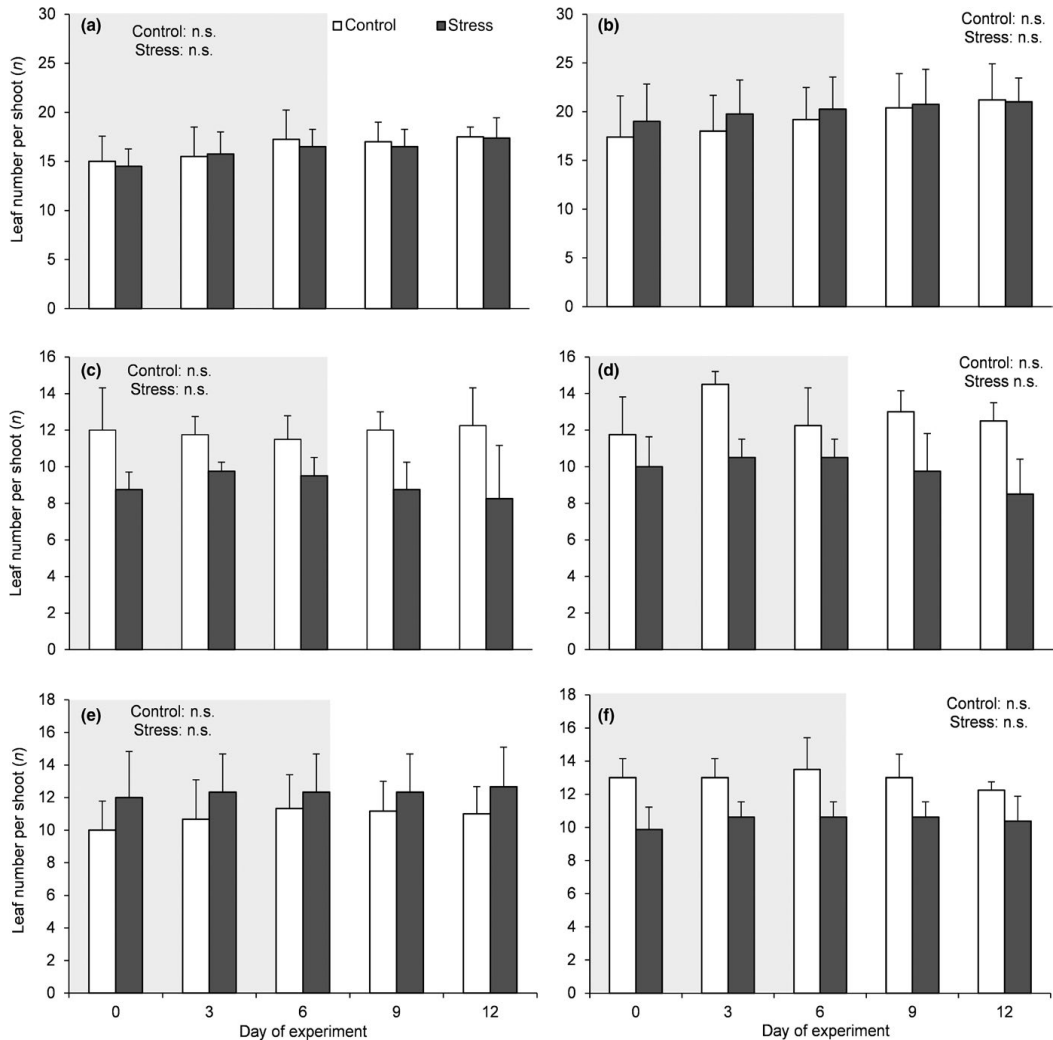
FIGURE 2 Mean values (±*SD*) of leaf number per shoot in: (a–b) *Cistus monspeliensis* from Sardinia and from southwest Spain, (c–d) *C. creticus* subsp. *eriocephalus* from Sardinia and from the Italian mainland, and (e–f) *C. salvifolius* from southwest Spain and from Sardinia. Differences were considered only within treatment (Control and Stress, white and gray bars, respectively) by considering sampling day as main factor (one-way ANOVA, *p* ≤ .05, *n* = 10). Shaded areas indicate the days of withholding irrigation

recovery in these species after drought stress suppression (Puglielli, Redondo-Gómez, et al., 2017). However, the underlying mechanisms of such response are beyond the scope of the present work.

In terms of the considered parameters, F_v/F_m, a measure of the degree of photoinhibition (Oliveira & Peñuelas, 2002), was the only one which was decoupled from the PI_{stress}–$PI_{recovery}$ trade-off. This is not surprising, as both physiological and morphological mechanisms are known to be less effective in allowing F_v/F_m recovery after drought stress in *Cistus* spp. (Correia & Ascensão, 2017). Otherwise, the results suggest that changes in F_v/F_m do not represent a major

factor in drought stress-induced photosynthesis depression as gas exchange parameters widely recovered after restoring irrigation, according to the result obtained for other Mediterranean species (Catoni et al., 2017; Flexas et al., 1998). Moreover, low Ψ_{Pd} does not always lead to severe reduction of F_v/F_m in *Cistus* leaves (Grant et al., 2014). We suggest that F_v/F_m response may be mostly related to very long-term drought events in these species. However, as F_v/F_m changes in *Cistus* spp. are also buffered by structural modifications of the light-harvesting complexes in leaves formed in different environmental conditions (Grant et al., 2014; Puglielli, Cuevas
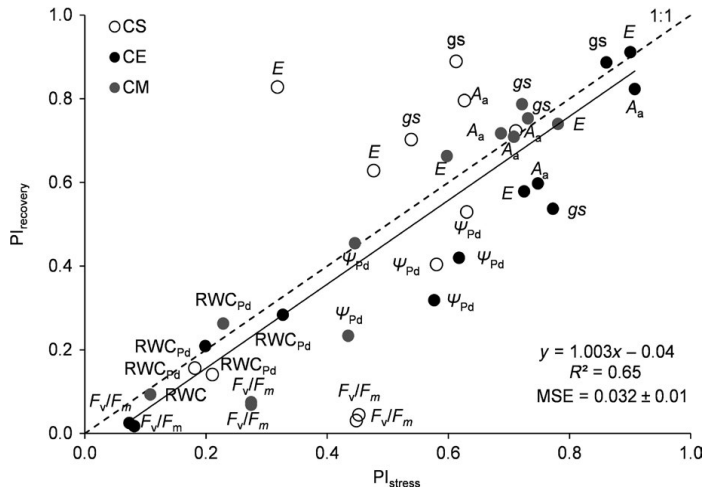
FIGURE 3 Relationship between the phenotypic plasticity index measured in response to stress (PI_{stress}) and that to recovery ($PI_{recovery}$) for the considered physiological traits. Values per each physiological parameter and provenance were used as experimental units ($n = 36$). The fitted equation, its R^2, the 1:1 line as well as the mean square error (MSE) obtained after cross-validation (see Materials and Methods section) are shown. A_a, net photosynthesis per unit leaf area; gs, stomatal conductance; E, transpiration; F_v/F_m, maximum quantum efficiency of PSII photochemistry; Ψ_{Pd}, predawn water potential; and RWC_{Pd}, predawn relative water content

Román, et al., 2017), F_v/F_m interpretation can be much more complex. At any rate, our statements on the absence of a significant photoinhibition are confirmed by the lack of leaf shedding in stressed plants. Leaf shedding during summer in *Cistus* spp. is in fact caused by chronic photoinhibition, which is usually detected through deep F_v/F_m reduction (Werner et al., 1999, 2002).

In conclusion, our results confirmed the hypothesized trade-off between the extent of the plastic response to stress and that to recovery. The trade-off contributes in explaining the profit, more than the cost, of a higher physiological plasticity in response to short-term stress imposition for *Cistus* spp. Based on our evidence, the highlighted behavior is involved in avoiding premature leaf shedding in short-term stressful events. This may also represent a good strategy to hold the more costly summer leaves (compared to winter leaves, Puglielli G. & Varone L. unpublished) in order to maintain relatively high productivity to face more prolonged drought events. Species that are constrained to maximize their performance during the favorable period, such as *Cistus* spp., have to sustain a higher cost in terms of plasticity when drought occurs. We demonstrated that such cost is paid back in terms of a higher short-term plasticity in the recovery.

However, the relationship PI_{stress} versus $PI_{recovery}$ seems to be parameter-specific and therefore deserves to be tested including a wide range of species and traits in order to get a deeper overview of the costs and/or profits arising from a greater plasticity in response to stress factors. This is particularly important considering that climate change will modify the frequency of stressful events (Giorgi & Lionello, 2008), calling for major attention on the resilience mechanisms of species inhabiting environments threatened by climate change, such as the Mediterranean Basin.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, drafted the article and revised it critically for important intellectual content, and gave final approval of the version to be published.

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Chapter 5

General Discussion

The results highlight important aspects of the adaptive strategies of the three *Cistus* species. In particular, the mechanisms by which these species cope with air temperature changes during the early growth stage (Chapter 2) and with stress factors (in winter and summer, Chapter 3 and 4, respectively) were described, as well as the provenance-dependent patterns of response.

The findings are strengthened by considering that *Cistus* plants from different provenances were cultivated under common garden conditions thus excluding any environmental confounding effect. Moreover, the results are relative to the first two years after germination which are particularly important considering that *Cistus* spp. are obligate seeders (Moreira and Pausas 2012; Pausas and Schwilk 2012), meaning that they rely on seedling recruitment after disturbance (Pausas and Keeley 2014). Debate exists on the germination cues necessary to allow *Cistus* seed germination (Troia and Laguna 2015). Many Authors (Roy and Sonie 1992; Pela et al. 2000; Olmez et al. 2007a,b; Delgado et al. 2008; Tavşanoğlu and Çatav 2012) analyzed the role of fire in allowing *Cistus* seed scarification and subsequent germination. Nevertheless, high soil temperatures (e.g. 60°C) experienced by seeds at midday in summer under Mediterranean conditions, have been demonstrated to be otherwise effective (Troia and Laguna 2015). In fact, *Cistus* spp. seed germination is related to variable and open habitats (Troia and Laguna 2015) and high density stands of *Cistus* seedlings rise after germination (Correia and Ascensão 2017). Seedlings then have to cope with environmental factor variations during the establishment phase following germination. This is crucial for determining a successful recovery of a given population (Quintana et al. 2004) due to two main factors: (i) a narrow temporal (and temperature) window in which the species maximize growth after germination following a disturbance (Quintana et al. 2004) and (ii) the stress pressure exerted by winter low temperature and summer drought during seedling establishment (Quintana et al. 2004; Cochrane et al. 2015; El Aou-Ouad et al. 2015). Summer drought and low winter temperatures associated to high radiation pose serious limitations to the physiological performance of plant species inhabiting the Mediterranean basin (Gratani and Varone 2004; Catoni et al. 2012; Flexas et al. 2014; Niinemets and Keenan 2014).

As a result, plants have to maximize growth and physiological performance in a relatively short favorable period. Accordingly, during the favorable period, following heat-induced germination (see Materials and Methods section in Chapter 2), *Cistus* seedlings maximize growth in a short time reaching their asymptotic height in approximately 90 days with significant differences in the RGR_{max}. The observed variations of RGR_{max} were due to changes in mean daily air temperature (T_M) for each of the considered provenances. In particular, the results highlighted that if RGR_{max} is reached at a higher temperature, then the temperature responsiveness of RGR_{max} is higher and the asymptotic height lower. Temperature is a major factor affecting plant distribution since it affects a

range of enzymatically catalyzed and membrane-associated processes and therefore plant allocation patterns (Lambers et al. 1998). Therefore, the highlighted trade-off well reflects the effects of the high temperature on plant size since the time to supply the assimilated to the growing organs decreases with increasing temperature resulting in an overall small size (Morison and Lawlor 1999). RGR_{max} is a key trait in predicting species distribution in different environments (Vile et al. 2006) and is genetically determined (Lambers and Poorter 1992). The results showed a different temperature responsiveness of RGR_{max} in the selected provenances. Considering that plant species in the natural environment are distributed across heterogeneous areas associated to climatic gradients, variations in environmental requirements and tolerances among populations is common (Moles et al. 2014; Cochrane et al. 2015). The obtained results are in line with these statements and reflect intraspecific adaptations to the sites of origin for *Cistus* spp. as a result of changes in the temporal (and temperature) window for seedling establishment. These results add a further evidence on the opportunistic strategies of *Cistus* spp. and the highlighted trade-off may be under selection.

Plant growth is the result of the optimal compromise between sink activity and resource acquisition (i.e. water and nutrients) (Cornelissen et al. 1992). Since in the experimental plan neither water nor nutrients were limiting, the observed trade-off can be indicative of the response ability of the considered species to temperature changes during the early growth stages. Such trade-off has, however, a cost in terms of seedling height. Given the functional role of plant height (Moles et al. 2009), a temperature increase may potentially alter the competitive ability of these species during the early growth stages according to their provenance.

Net photosynthetic rate decreases were observed in response to both low temperature in winter and drought in summer, according to previous studies (Larcher 2000; Gratani and Varone 2004; Zunzunegui et al. 2009; Flexas et al. 2014). Nevertheless, similar response patterns between provenances were highlighted. This result suggests that net photosynthesis (and related gas exchange parameters) may be subjected to a greater differentiation in the field (i.e. plastic traits). Even if little evidence is available to confirm this statement, this view is supported by differences in gas exchange parameters, such as stomatal conductance, found between *Cistus* spp. provenances in the field (Grant and Incoll 2005).

The results also showed interesting aspects concerning F_v/F_m and leaf morphological changes through the winter and spring season. In general, the behavior of F_v/F_m in response to low temperatures has aroused discordance in the literature relative to *Cistus* spp. In fact, both low (Zunzunegui et al. 1999, Oliveira and Peñuelas 2000, Grant and Incoll 2005) and high (García-Plazaola et al. 2000, Zeliou et al. 2009) photochemical efficiency has been found in *Cistus* leaves in

response to low temperatures (Flexas et al. 2014). In addition, Gratani and Bombelli (1999), Oliveira and Peñuelas (2000) and Grant and Incoll (2005) showed an increase of LMA in response to low winter temperatures, which was ascribed as some degree of leaf hardening by Oliveira and Penuelas (2004). Based on their results and literature evidences, Oliveira and Penuelas (2004) speculated that *Cistus* spp. could rely on the production of distinct functional leaf types over the year to also reduce the need for antioxidant mechanisms, thus stressing a similar previous hypothesis from García-Plazaola et al. (2000). The experimental design implemented in Chapter 3 allowed to provide compelling evidence on such hypotheses. The results highlighted that these species rely on the formation of functionally different leaves through winter up to spring. In particular, changes in F_v/F_o are the main targets of such changes and buffer changes in F_v/F_m (see Chapter 3 for further discussion). Moreover, F_v/F_o variations were also provenance dependent in two of the three considered species reflecting that winter chilling may exert a selective pressure on photochemical parameters for *Cistus* spp. according to their provenance. Such conclusion is supported by the F_v/F_m variability found between *Cistus albidus* populations in the field (Grant and Incoll 2005). These results can also contribute to explain the disagreement on the F_v/F_m behavior in *Cistus* spp. in response to chilling. Furthermore, the modulation of the size and the number of reaction centers, as reflected by F_v/F_o changes (Israr et al. 2011), through climatically different conditions results in a low necessity for energy expenditures in modulating biochemical photo-protective mechanisms (which require ATP consumption). This is supported by the lack of morphological control of the photosynthetic process (on a mass basis) under suboptimal air temperatures. Considering that these are species with a short leaf life span, it is not worth reducing mass-based net photosynthesis with increased LMA under unfavorable conditions. In fact, a lower instantaneous productivity due to increased leaf stiffness plus a short leaf duration would result in a low total productivity over the leaf life span, making it unlikely for a leaf to maintain the costs of the leaves' economics (Reich et al. 1997, Ackerly 2004, Saura-Mas et al. 2009). The observed lack of coordination between morphology and physiology was not otherwise a consequence of stomatal limitations to the photosynthetic process, which are known to constrain leaf structure-function relationships (Niinemets 2015). This is attested by the lack of relationship LMA-A_m even when stomatal conductance was not as low as under chilling (Chapter 2). When favorable conditions were restored, then the leaf structure-function relationships were in accordance with the leaf economic theories (Wright et al. 2004) and reflected an investment related to species-specific leaf turnover strategies (Chapter 3).

The results partially fill a gap of knowledge as the analysis of medium-term adjustments in leaf physiology and morphology and their relationship provides a clear evidence on the existence of

functionally different leaves within the same leaf type (i.e. winter leaves in this case). The ecological advantage is represented by *Cistus* spp. ability to adapt leaf physiology and morphology according to environmental condition changes in order to maximize resource acquisition by minimizing leaf construction costs. Overall, the reported evidence agrees with the pioneer character of *Cistus* spp. and highlights an effective (and less costly) functional resilience mechanism during the transition from suboptimal to favorable environmental conditions, extending previous findings from literature (e.g. Werner and Maguas 2010).

Cistus spp. ability to rapidly recover after stress imposition has long been recognized (Correia and Ascensão 2017), in agreement with the findings reported in Chapter 4. So far, the consequences of drought have been described in plants in terms of phenotypic plasticity, evolutionary adaptation and genetic canalization (Díaz Barradas et al. 1999; Quero et al. 2006; Gimeno et al. 2009; Ramírez-Valiente et al. 2010; Lamy et al. 2011). However, few studies investigated adaptive responses of plants from different provenances (e.g. Gratani et al. 2003; Ramírez-Valiente et al. 2010; De la Mata et al. 2014; Varone et al. 2016; Santiso and Retuerto 2016). The drought and recovery responses of the three *Cistus* spp. shown in Chapter 4 were not dependent on the provenance. Furthermore, the drought and recovery responses, quantified via phenotypic plasticity index *sensu* Valladares et al. (2000), showed a positive trade-off across species and provenances. Such result highlights a convergence in the response to drought for the considered *Cistus* spp. A similar conclusion was drawn by Santiso and Retuerto (2016) which highlighted a high degree of plasticity in response to drought independently of the provenance for the Mediterranean *Arbutus unedo* grown under common garden conditions.

The observed convergence in drought and recovery responses can also be explained by considering the seasonal trend of photosynthesis for *Cistus* spp. When environmental conditions are favorable, *Cistus* spp. show higher photosynthetic rates than those recorded for evergreen sclerophyllous (Harley et al. 1987, Gratani and Bombelli 1999, Gratani and Varone 2004, Gratani and Varone 2006, Gulias et al. 2009, Gallé et al. 2011, Vilagrosa et al. 2014, Correia and Ascensão 2017, Gratani et al. 2017). Nevertheless, the higher the photosynthetic rate under favorable conditions is the higher its decrease when stress factors occur (Lortie and Aarssen, 1996; Valladares et al., 2007; Bongers et al. 2017; Zhang et al. 2017), posing a cost in terms of physiological plasticity for *Cistus* spp. (Lortie and Aarssen, 1996; Valladares et al., 2007). Despite this, a fast recovery of physiological parameters generally occurs after the first autumn rainfall (Werner et al. 1999, 2002; Gratani and Varone 2004; Zunzunegui et al. 2009) and after restoring irrigation in pot experiments (Gallé et al. 2011; Puglielli et al. 2017a). The highlighted trade-off is therefore indicative of the instantaneous response capacity of *Cistus* spp. to stress suppression reflecting that

the physiological mechanisms during recovery are crucial to offset the costs of a high short-term physiological plasticity in response to drought, in agreement with the results of Zunzunegui et al. (2009) for the semi-deciduous *Halimium halimifolium*.

The expected relationship between stress and recovery responses had not been previously quantified. Moreover, it is widely recognized that species can differ in their drought response in terms of both decline and recovery of the physiological parameters, in particular stomatal conductance and photosynthetic rate (Chaves et al. 2009). As an example, a faster photosynthesis than stomatal conductance recovery upon re-watering after drought-induced suppression has been documented for rehydrated plants after exposure to different degrees of drought stress (Gallé et al. 2007; Perez-Martin et al. 2014; López-Jurado et al. 2016, Puglielli et al. 2017a). Gallé et al. (2007) suggested that such response might be species-specific or stress-specific. In the experiment, the recovery was considered to have occurred after a stomatal conductance threshold of 0.2 mol m^{-2} s^{-1} considering that such value is often encountered for well-watered plants of *Cistus* spp. in pot experiments (Gallé et al. 2011; Puglielli et al. 2017a). This means that at that given stomatal conductance, net photosynthesis recovery could not have occurred. However, the presented trade-off held across gas exchange parameters, species and provenances and was also independent of the species-specific strategies to cope with drought. Such considerations significantly increase the outcome of the study.

F_v/F_m was the only parameter decoupled from the highlighted trade-off due to a minor response of this parameter to short-term drought imposition. Werner et al. (1999, 2002) demonstrated that chronic photoinhibition (i.e. F_v/F_m does not recover at predawn when compared to midday values) is responsible for leaf shedding in *Cistus* spp. and usually, when water potential and stomatal conductance to CO_2 are very low, *Cistus* leaves show a strong photoinhibition (i.e. F_v/F_m drop). Leaf shedding surely represents an advantage during long-term drought events due to the necessity to reduce the transpiring surface and to down-regulate carbon assimilation (Correia and Ascensao 2017). On the contrary, an unpredictable drought event with a short duration should not result in early leaf shedding. During the experiment, leaf shedding was not observed for stressed plants.

It can be argued that the fast recovery capacity after restoring irrigation, together with a certain degree of F_v/F_m resistance to short-term drought, allows *Cistus* spp. to avoid the progression of chronic photoinhibition. As a consequence, early leaf shedding might be impeded during short-term drought events and this statement is supported by considerations on leaf construction costs. *Cistus* spp. display a specific leaf type to face summer drought, the so-called summer leaves. Such leaf type is emitted during late spring and is characterized (on average) by a greater LMA, leaf

thickness, tissue density and, in general, by a different carbon fixation strategy (Gratani and Bombelli 1999; Aronne and De Micco 2001; Catoni et al. 2012). Summer leaf features suggest that they are more costly than winter leaves (i.e. lower LMA, leaf thickness and tissue density), in agreement with the results obtained by Puglielli and Varone (unpublished). Therefore, the previous considerations suggest that it is worth retaining summer leaves by avoiding leaf shedding during short-term drought. This is an advantageous short-term strategy in allowing a positive carbon balance to be maintained until the onset of prolonged drought periods.

Apart from the main aspects discussed so far, it is interesting to notice that species-specific physiological patterns of response to stress, whether chilling or drought, were highlighted. In particular, it was found that *C. monspeliensis* and *C. creticus* subsp. *eriocephalus* showed a similar pattern of response to stress factors while *C. salvifolius* was always found to respond differently (see Chapters 3 and 4 for further discussion). Such differences are supported by the positioning of these three species in the tertiary CSR strategy scheme recently proposed by Pierce et al. (2017). In fact, while *C. monspeliensis* and *C. creticus* subsp. *eriocephalus* are only classified as stress tolerators (i.e. S), *C. salvifolius* is characterized by a S/CS strategy. The results, from a physiological point of view, can contribute to highlight the different response of *C. salvifolius* compared to the other species. Moreover, it should be considered that *C. salvifolius* is characterized by the most mesic distribution compared to *C. monspeliensis* and *C. creticus* subsp. *eriocephalus*, and it is the only studied species that can grow in the understory (see Zunzunegui et al. 2016 and Puglielli et al. 2017b for further discussion). It also colonizes a great variety of habitats (Farley and Mc Neilly 2000) and is the most widely spread species of the genus around the Mediterranean Basin (Puglielli et al. 2017b). Therefore, its wider geographical and ecological distribution in respect to the other two species somehow justifies its different physiological response to the main Mediterranean environmental cues, even if more studies are needed to clarify this issue.

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Chapter 6

Conclusions

Overall, the results highlight important aspects of *Cistus* spp. adaptive strategies to Mediterranean stress factors. The trade-offs and mechanisms presented fit the pioneer characters of these species resulting from their regenerative strategy as well as their ability to track favorable environmental periods. Moreover, the effect of provenance was found only for photochemical parameters under winter chilling and during early growth.

In particular, the different functional characteristics at the early growth stages (i.e. differences in RGR_{max}) suggest that the duration of the favorable period, in terms of daily temperature changes, may exert a selective pressure for *Cistus* spp. The results also confirm that the short leaf longevity associated to low construction costs confer advantage to *Cistus* spp. in harsh habitats. Such characteristics determine how these species adapt leaf morphology and physiology to changes in prevailing environmental conditions. This is achieved by the production of functionally different leaves and was particularly evident during the transition from suboptimal air temperatures (under chilling) to more favorable environmental conditions (early spring). Intraspecific differences in photochemical parameters support this conclusion and also suggest that winter chilling can represent a selective pressure for photochemical parameters.

A marked plasticity of response to environmental changes for these species was highlighted. *Cistus* spp. plastic behavior was well reflected during short-term drought and subsequent recovery. In particular, resilience ability equated the stress induced physiological downregulation under stress. This result confirms that in the short-term such species can resume their activity during moderately favorable periods. Such strategy can confer the advantage of delaying leaf shedding of the more costly summer leaves (compared to winter leaves), which otherwise occurs under prolonged drought stress conditions. Furthermore, a general lack of intraspecific differences was found, suggesting a functional convergence across provenances in response to short-term drought.

In general, it is evident that *Cistus* spp. are affected by stress imposition but they can recover as soon as favorable conditions are restored. This is achieved through their ability to respond to climate unpredictability by adjusting, both in the short- and mid-term, their photosynthetic organs, morphologically and/or physiologically, in order to enhance their performance during favorable climatic conditions.

In view of the results, it is possible to hypothesize how the opportunistic behavior of *Cistus* spp. will constrain the species response to global climate change.

According to Giorgi and Lionello (2008), the projections of climate change in the Mediterranean Basin indicate that temperature rise and extreme temperature events will occur. As a result, the favorable seasonal periods may be shortened and the thermal amplitudes over the year

may change stochastically. This puts *Cistus* spp. in a critical position. In particular, steep changes in terms of temperature during the favorable period can affect foliar structure-function relationships. The likely direct consequence would be an alteration of leaf turnover strategies with further consequences on the efficiency of nutrient re-translocation, a well-known process in *Cistus* spp. (Milla et al. 2004; Simões et al. 2012; Dias et al. 2012). This can ultimately affect their resilience ability due to limited resource availability for the leaves that will be emitted. Considering that litter fall is also one of the main input of organic matter and nutrients to the soil, this may have consequences for nutrient recycling and ecosystem functioning (Correia and Ascensão 2017).

According to the results of the growth analysis, an increase in air temperature during the favorable period may result in a reduced height due to a narrower temperature (and temporal) window in which to maximize RGR, with predictable consequences in terms of competitive ability.

On the contrary, concerning the drought response in the short-term, the highlighted trade-off suggests that *Cistus* spp. may be able to face the unpredictability of rain distribution more than the forecasted increase in air temperature. Nevertheless, if the duration and intensity of drought increases, then *Cistus* spp. have to cope with the inevitable photoinhibition and leaf shedding which has already been defined as a '*winning strategy*' to face climate change (Correia and Ascensão 2017).

In conclusion, the evidence strongly supports the idea that, at least during the first two years of growth, the opportunistic strategy of *Cistus* spp. is risky if favorable seasonal environmental conditions change markedly or too fast. This can threaten their competitive abilities with wider consequences in terms of vegetation succession, nutrient recycling and therefore Mediterranean ecosystem functioning.

The characterization of the response of plant species to the major targets of climatic change is important in order to highlight mechanisms that can be further investigated both experimentally and in the field. The findings of this thesis provide a conceptual framework, in terms of experimental designs, traits to be included and mechanisms that deserve to be applied by including other *Cistus* spp. in the field in order to extend the results at the genus level.

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List of publications *(* indicates GP as the Corresponding Author)*

Thesis chapters

(1) ****Puglielli G**, Spoletini A, Fabrini G, Gratani L (2017). Temperature responsiveness of seedlings maximum relative growth rate in three Mediterranean *Cistus* species. *Journal of Plant Ecology* 10, 331–339. *(**IF 2016**: 1.754).*

(2) ****Puglielli G**, Cuevas Román FJ, Catoni R, Moreno Rojas JM, Gratani L, Varone L (2017). Provenance effect on carbon assimilation, photochemistry and leaf morphology in Mediterranean *Cistus* species under chilling stress. *Plant Biology* 19, 660–670. *(**IF 2016**: 2.106).*

(3) ****Puglielli G**, Catoni R, Spoletini A, Varone L, Gratani L (2017). Short-term physiological plasticity: trade-off between drought and recovery responses in three Mediterranean *Cistus* species. *Ecology and Evolution* (in press). *(**IF 2016**: 2.440).*

Other publications pertinent to the PhD project

(1) **Puglielli G**, Redondo-Gómez S, Gratani L, Mateos-Naranjo E (2017). Highlighting the differential role of leaf paraheliotropism in two Mediterranean *Cistus* species under drought stress and well-watered conditions. *Journal of Plant Physiology* 213, 199–208. *(**IF 2016**: 3.121).*

(2) ****Puglielli G**, Varone L, Gratani L, Catoni R (2017). Specific leaf area (SLA) variations drive acclimation of *Cistus salvifolius* in different light environments. *Photosynthetica* 55, 31–40. *(**IF 2016**: 1.507).*

(3) Gratani L, Varone L, Crescente MF, Catoni R, Ricotta C, **Puglielli G** (2017). Leaf thickness and density drive the responsiveness of photosynthesis to air temperature in Mediterranean species according to their leaf habitus. *Journal of Arid Environments* (in press). *(**IF 2016**: 1.835).*

(4) ****Puglielli G**, Varone L (2018). Inherent variation of functional traits in winter and summer leaves of seasonal dimorphic *Cistus* species: evidence of a ´within leaf cohort` spectrum. Submitted on *AoB Plants*.

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Other publications

(1) **Puglielli G**, Crescente MF, Frattaroli AR, Gratani L (2015). Morphological, anatomical and physiological leaf trait plasticity of *Sesleria nitida* in open vs shaded conditions. *Polish journal of Ecology* 63, 10–22. (**IF 2015**: *0.500*).

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Project coordination

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