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Are red leaf phenotypes more or less fit? The case of winter leaf reddening in *Cistus creticus*

Constantinos Nikiforou, Konstantina Zeliou, Velissarios-Phaedon Kytridis, Alexandra Kyzeridou, Yiannis Manetas*

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

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ABSTRACT

Winter leaf reddening occurs in some plants as a response to the combination of cold temperatures and high light. Hence, a protective function against photoinhibition of photosynthesis has been suggested. However, it is unknown whether the leaf anthocyanic trait confers long term benefits to the plant and to that aim parameters related to fitness were measured.

We took advantage of intra-species variation in the expression of the red leaf character displayed by the Mediterranean shrub *Cistus creticus*, a field of which during winter becomes a mosaic of green and red individuals under apparently similar environmental conditions. The red individuals are known to be more sensitive to winter photoinhibition of photosynthesis, hence anthocyanins might serve a compensatory function through light screening and/or detoxifying reactive oxygen species. If anthocyanins are indeed beneficial in this regard, this might be reflected in altered growth and reproductive output of red compared to green individuals.

Both phenotypes displayed similar photosynthetic performance indices before and after the winter red period, yet reds suffered a considerable drop in this parameter concomitant with anthocyanin accumulation. This photosynthetic inferiority was irregularly linked to growth, since red plants produced fewer new leaves during the following spring, yet shoot relative growth rate was higher. Moreover both phenotypes displayed similar flower numbers, pollination success and seed yield, mass and germinability.

As judged by the similar final reproductive output, vulnerability to the winter stress does not render the red phenotype less fit, nor anthocyanin accumulation render it more fit. Moreover, the photosynthetic inferiority of the red phenotype, although linked to slightly reduced leaf number, it was not limiting for reproductive effort and success.

Regardless of function, winter leaf redness in *C. creticus* may indicate photosynthetically weak individuals. However, neither a fitness cost nor benefit of anthocyanins can inferred in this system.

1. Introduction

Anthocyanins advertise the presence of flowers to pollinating animals. Yet, in some plants and under some circumstances, they are accumulated in leaves, masking the green chlorophyll color. We may distinguish between developmentally controlled redness, occurring either in young, developing or old, senescing leaves and environmentally induced in mature leaves under stress (Manetas, 2006; Archetti et al., 2009). Anthocyanins absorb visible radiation without being photosynthetic. Accordingly, they compete with photopigments for photon capture and as such, may entail a photosynthetic cost to the leaf due to lost photons under sub-saturating photosynthetic photon flux densities. Since the leaf anthocyanic trait has been preserved in most plants (especially angiosperms), it has been argued that a benefit is afforded to red leaves, compensating for the combined construction and photosynthetic costs. Many hypotheses have been proposed for a function of leaf anthocyanins, yet a consensus has not being reached.

Ecological hypotheses link the anthocyanic trait to insect herbivory in one way or another. Leaf redness has been considered as a signal indicating a high defensive potential, provided that insects can perceive the red signal and avoid the bearer. Alternatively, it may be considered as a camouflage resulting from the masking of green leaf color which is usually perceived by folivorous insects (Stone, 1979; Archetti, 2000; Hamilton and Brown, 2001; Archetti and Brown, 2004; Archetti et al., 2009). In a third variant, the red leaf color is considered to undermine the insect camouflage, since a (usually green) folivorous insect is better seen by its predator when feeds on a red object (Lev-Yadun et al., 2004). There are sporadic reports supporting the ecological hypotheses for young or

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* Corresponding author. Tel.: +30 2610 997411x96; fax: +30 2610 997411.
E-mail address: y.manetas@upatras.gr (Y. Manetas).

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senescing red leaves (Furuta, 1986; Numata et al., 2004; Archetti and Leather, 2005; Karageorgou and Manetas, 2006). However, the significance of these hypotheses for the transient accumulation of anthocyanins during winter is apparently weakened, since folivorous insect activity is considerably less during this period.

Among physiologists, the most prominent hypothesis is that of photoptotection against excess light, especially when other environmental stresses perturb the delicate balance between light capture and its use for CO_2 assimilation. It is argued that when all the available biochemical and behavioral measures against photoinhibition are exhausted, anthocyanins may come to play by affording a light screen (reducing excitation pressure on photopigments) and/or detoxifying reactive oxygen species produced by chlorophyll over-excitation (Lee and Gould, 2002; Steyn et al., 2002; Manetas, 2006). The hypothesis is reasonable since those environmental stresses that increase the risk of photoinhibitory damage also induce leaf redness in some plants, but not in the majority of them (Lindoo and Caldwell, 1978; Christie et al., 1994; Hodges and Nozzolillo, 1996). However, recent experimental approaches gave conflicting results, some cases confirming (Feild et al., 2001; Hughes et al., 2005; Hughes and Smith, 2007a,b) and others rejecting the hypothesis (Burger and Edwards, 1996; Lee et al., 2003; Kyparissis et al., 2007; Esteban et al., 2008). Moreover, it has being argued that neither the optical properties of leaf anthocyanins are ideal for the sunscreen function, nor their intracellular or tissue location always appropriate for the anti-oxidative function (Kytridis and Manetas, 2006; Manetas, 2006).

Regardless of function, one could argue that if anthocyanins are indeed beneficial, this could be manifested in enhanced vigor and fitness of the red phenotypes under field conditions. In this study, we approach for the first time the question of fitness in red- and green-leaf phenotypes, using the Mediterranean shrub *Cistus creticus* as a test system. The system is advantageous since some individuals of *C. creticus* become red during winter, while neighboring individuals, under apparently similar light exposure and on the same soil, remain green. Repeated observations with tagged individuals since 2002 indicated that the red character during winter was stable, i.e. the same individuals become red each year (Kytridis et al., 2008). However, this apparent advantage is confounded by the finding that the red phenotype of *C. creticus* exhibits also lower light-saturated PSII yields and pool sizes of photoprotective xanthophylls during winter and lower levels of leaf nitrogen throughout the year (Kytridis et al., 2008; Zeliou et al., 2009). Hence, any link between leaf redness and plant vigor should be seen within the context of these co-existing differences among the corresponding phenotypes.

Assessment of vigor and fitness was made by measuring photosynthetic performance, growth and reproductive output. Photosynthesis plays a central role in energy and carbon acquisition and biomass accumulation and, indirectly, to fitness. Aspects of photosynthetic function can be assessed through in vivo chlorophyll fluorescence measurements which are accurate reliable, rapid and non-invasive (Strasser et al., 1995). They are based on the analysis of transients in chlorophyll fluorescence rise after closing all PSII reaction centers with sudden illumination. Since PSII is the target of various environmental stresses (Long et al., 1994), the assessment of its functionality is pivotal in the location of vulnerable individuals or populations. In this investigation, a newly proposed parameter, the so-called photosynthetic performance index (PI) is used. PI encompasses both structural and functional attributes of PSII, and it is much more sensitive as a stress indicator than the frequently used maximum PSII yield (as $F_V/F_{M,}$ see Strasser et al., 2004). It has been shown to be positively correlated to CO_2 assimilation rates (van Heerden et al., 2003), and proposed as an index for the assessment of tree quality (Hermans et al., 2003).

Although photosynthesis and growth are proxy (indirect) measures of plant fitness, the ultimate indicator is reproductive success, i.e. the ability of the individual to produce a satisfactory and viable seed yield. Reproductive parameters display species-specific phenotypic plasticity and are responsive to stress to a varying degree (Marshall et al., 1986; Sultan, 2001). In the present study, we use all the above indirect and direct criteria of fitness in the red/green leaf debate.

2. Materials and methods

2.1. Plant material and sampling area

C. creticus L., Cistaceae is an evergreen Mediterranean shrub pioneering post fire sites. Our study site suffered a wild fire in 1989 and at present it is dominated mainly by *C. creticus*, *C. salvifolius* and regenerating evergreen sclerophyll and pine trees. The study area (38.14°N, 21.44 °E, 250 m a.s.l.) covers the South facing foot of a hill.

Twenty six apparently similar, fully exposed individuals (14 green, 12 red) were tagged during winter 2006, to monitor reproductive parameters in the subsequent spring and summer period.

In 2008, 29 individuals (different from those used in 2006, 15 green, 14 red) were used to confirm previous observations on reproductive parameters and concurrently monitor shoot growth and leaf production during spring/early summer. In addition, photosynthetic performance and leaf anthocyanins were measured at the indicated sampling dates from December 2007 (i.e. 2 weeks before reddening) and up to August 2008, encompassing both the red and the green period of the year. For destructive measurements (i.e. photosynthetic performance and anthocyanins), the removed leaves were less than 2% of the current leaf population.

Mean monthly temperatures for winter months of the 2 sampling years were10.3, 7.1 and 9.2 °C for December 2005, January and February 2006 and 10.2, 10.5 and 10.7 °C for December 2007, January and February 2008. Corresponding 10 years (1997–2006) means were 11.1, 9.5 and 9.9 °C, respectively. Data were kindly given by the Regional Institute of Plant Protection, located 3 km from the sampling site.

2.2. Photosynthetic performance (PI)

Six mature leaves from each plant were harvested at predrawn of each sampling date, put in air-tight plastic envelopes lined internally with moist filter paper and kept in the dark for 1 h until measurement. Sampling dates encompassed both the “green” and the “red” period of the year, as shown in the corresponding Fig. 1. Leaves begin to senesce by mid spring and completely drop by mid June. Such leaves were not sampled for PI, hence the May measurement was done on mature leaves of current year growth. Chlorophyll a fluorescence transients were induced by red light (peak wavelength at 650 nm, 3000 μmol photons $m^{-2}\,s^{-1}$) given by a bank of three light-emitting diodes and fluorescence was captured by a Hansatech (Handy-PEA, Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK) analyzer with appropriate data acquisition rates. The photosynthetic performance index (PI) was calculated from fluorescence values at cardinal points of the fluorescence versus time kinetics as

$$\mathrm{PI} = \left[\frac{1-(F_0-F_M)}{4(F_K-F_0)/(F_J-F_0)}\right] \times \left[\frac{(F_M-F_0)}{F_0}\right] \times \left[\frac{(F_M-F_J)}{(F_J-F_0)}\right],$$

where F_0, F_K and F_J the fluorescence yields at 20 μs, 300 μs and 2 ms, respectively, while F_m is the final maximum fluorescence