Lazaroa
ISSN: 0210-9778

EDICIONES
COMPLUTENSE

http://dxd.oi.org/10.5209/LAZAROA.51795

Ecophysiology of *Adonis distorta*, a high-mountain species endemic of the Central Apennines

Valter Di Cecco[1], Rosangela Catoni[2], Giacomo Puglielli[2], Luciano Di Martino[3], Anna Rita Frattaroli[1] & Loretta Gratani[2]

Received: 1 February 2016 / Accepted: 23 September 2016

Abstract. Morphological, anatomical and physiological plant and leaf traits of *A. distorta*, an endemic species of the Central Apennines on the Majella Massif, growing at 2675 m asl, were analyzed. The length of the phenological cycle starts immediately after the snowmelt at the end of May, lasting 128±10 days. The low *A. distorta* height (H_{max}= 64±4 mm) and total leaf area (TLA= 38±9 cm^2) associated to a high leaf mass area (LMA =11.8±0.6 mg cm^{-2}) and a relatively high leaf tissue density (LTD= 124.6±14.3 mg cm^{-3}) seem to be adaptive traits to the stress factors of the environment where it grows. From a physiological point of view, the high *A. distorta* photosynthetic rates (P_N =19.6±2.3 µmol m^{-2} s^{-1}) and total chlorophyll content (Chl_{a+b}= 0.88±0.13 mg g^{-1}) in July are justified by the favorable temperature. P_N decreases by 87% in September at the beginning of plant senescence. Photosynthesis and leaf respiration (R_D) variations allow *A. distorta* to maintain a positive carbon balance during the growing season becoming indicative of the efficiency of plant carbon use. The results could be an important tool for conservation programmes of the *A. distorta* wild populations.

Keywords: *Adonis distorta;* leaf nitrogen content; photosynthetic nitrogen use efficiency; photosynthesis.

[es] Ecofisiología de *Adonis distorta*, especie de alta montaña endémica de los Apeninos centrales

Resumen. Se han estudiado los rasgos morfológicos, anatómicos y fisiológicos de hojas de la especie endémica *Adonis distorta* en el macizo de Majella, Apeninos centrales. La especie crece a 2675 m de altitud y la duración del ciclo fenológico se inicia inmediatamente después de la fusión de la nieve a finales de mayo, con una duración de 128±10 días. El pequeño tamaño de la planta (H_{max}= 64±4 mm) y el área foliar total (TLA= 38±9 cm^2) asociada a una elevada masa foliar (LMA= 11.8±0.6 mg cm^{-2}) y una relativamente alta densidad titular de la hoja (LTD= 124.6±14.3 mg cm^{-3}) parecen ser los rasgos de adaptación a los factores de estrés del entorno en el que crece. Desde un punto de vista fisiológico, las altas tasas fotosintéticas (P_N =19.6±2.3 µmol m^{-2} s^{-1}) y el contenido total de clorofila en julio (Chl_{a+b}= 0.88±0.13 mg g^{-1}), se justifican por la temperatura más favorable de ese mes. P_N disminuye en un 87% en septiembre al inicio de la senescencia de la planta. la fotosíntesis y la respiración en la hoja (R_D) presenta variaciones que permiten a *A. distorta* a mantener un balance positivo de carbono durante la estación de crecimiento, siendo indicativo de la eficiencia del uso del carbono por las plantas. Los resultados podrían ser una herramienta importante para los programas de conservación de las poblaciones silvestres de *A. distorta*.

Palabras clave: *Adonis distorta;* contenido en nitrógeno de la hoja; eficiencia fotosintética; fotosíntesis.

Introduction

Models forecasting changes in species distribution as a function of climate scenarios for the 21st century predict a massive reduction in plant diversity (Dirnböck & *al*., 2011). Such effects could be more intense in mountain areas (Engler & *al*., 2011). In particular, as a consequence of global climate change, Mediterranean mountains will experience less

---

[1] Department of Life Health and Environmental Sciences, University of L'Aquila, Via Vetoio loc. Coppito, 67100, L'Aquila, Italy

[2] Department of Environmental Biology, Sapienza University of Rome, P.le A. Moro, 5 00185 Rome, Italy. Email: loretta.gratani@uniroma1.it (corresponding author).

[3] Majella Seed Bank. Majella National Park. loc. Colle Madonna. 66010, Lama dei Peligni (CH), Italy.

rainfall and more inter-annual variability in temperature and rainfall than other mountains in Europe (Giorgi & Lionello, 2008). Mediterranean mountains in southern Europe have been the site of a large number of speciation events (Martín-Bravo & *al*., 2010). Consequently, most of the species in the Apennines are relicts from past frost conditions (Sanz-Elorza & *al*., 2003). In particular, the entire Central-Apennines can be considered an arctic-alpine biogeographic archipelago since the nearest mountains with comparable elevations are more than 600 km away in the Alps (van Gils & *al*., 2012). Nevertheless, this genetic diversity could be significantly disturbed in the future by global climate change (Thuiller & *al*., 2005). Consequently, species extinction in the Mediterranean mountains could be higher than in the Boreal, Alpine or Atlantic regions (Thuiller & *al*., 2005). The alpine life zone is often considered a harsh environment, dominated by extreme climatic conditions and rough terrain, forcing plants to a high degree of specialization and adaptation (Körner, 2003). On steep slopes, ridges and depressions, the interplay between exposure and vegetation is leading to mosaics of life conditions (Körner, 2003). Moreover, alterations in the key factors governing alpine plant life, such as duration and depth of snow cover and temperature, will undoubtedly disrupt high altitude communities (Körner, 1999).

Adonis distorta Ten. is an endemic species of the Central Apennines growing on the main carbonatic massifs such as Majella, Gran Sasso, Velino, Sirente, Sibillini, Monti della Duchessa (van Gils & *al*., 2012). *A. distorta* is considered a characteristic feature of the high altitude landscape of the Majella (Di Pietro & *al*., 2008) where it grows up to 2,700 m a.s.l. The species is categorized as DD in the IUCN Red List, listed in the II and IV Annex of the Habitats Directive (Habitat Natura 2000), in the Appendix I of the Bern Convention and included in the Regional Red List of the Italian Flora as LR for Abruzzo, Marche and Umbria, and as VU for Lazio.

The main objective of this research was to analyze phenological, morphological, anatomical and physiological trait variations among plants of a wild population of *A. distorta* growing at 2675 m asl, on Majella National Park at the highest altitude of its distribution area. The knowledge for endemic and threatened species response to environmental factors in high mountain areas is essential for identifying wild population growth and persistence in the future. The monitoring of this wild population at the highest altitude of its distribution area in Abruzzo region is even more crucial in the light of climate change scenarios, due to the forecasted shifts of plant species at the upper edge of altitudinal range to escape rising temperatures. Moreover, the results could be an important tool for conservation programmes of the *A. distorta* wild populations.

Materials and Methods

Study area and climate

The study was carried out on an *A. distorta* population growing at 2675 m asl on Mount Focalone (42°62'18" N; 14°72'10" E) in Majella National Park (740 km^2; 130-2800 m asl, Central Apennines) at the highest altitude of its distribution area in the period May to October 2014.

The population was found on scree extending over an area of approximately 70 m^2 with a density of 11 plants m^{-2}. Phenological and morphological, anatomical and physiological plant and leaf traits were studied on 50 randomly selected plants. The limestone massif of Majella is the widest alpine area in the Apennines. It consists of a mountain ridge about 30 km in length, running in a north-south direction between 42°12'00" North and the 42nd parallel South, 32.5 km West of the Adriatic sea coast. A morphological peculiarity of the Majella is its dome shape which is flattened at the top and has very steep sides with deep gorges of glacial origin. The soil pH is 7.66±0.11, soil organic matter content (SOM) 2.55±0.28% and the ratio between carbon and nitrogen content (C/N) 9.98±0.33. The climate is characterized by a mean minimum air temperature (T_{min}) of –3.9±2.2°C (February), a mean maximum air temperature (T_{max}) of 22.3±0.1°C (July-August) and a mean annual air temperature (T_{mean}) of 7.6±6.5°C. Total annual rainfall is 1343 mm. Snow fall from December to April. During the study period, it snowed until the end of April. T_{min} of May was 5.5±1.5°C, T_{max} of July 22.3±2.1°C and total rainfall 415 mm (Data from the Meteorological Station of Passo Lanciano, Chieti, 42°18'62"; 14°09'87" for the years 2000–2013 and 2014).

Phenology and Plant traits

Phenological observations were made on selected plants in the period May-October. Vegetative activity (*i.e.* new leaf formation), flowering, fruiting and leaf senescence were monitored. Plant height was monitored weekly from the end of May (*i.e.* vegetative activity period) to the end of July when plants reached the maximum height (H_{max}, mm). The relative growth rate in height (RGR_H, mm mm^{-1} d^{-1}) was calculated by the equation: RGR_H= (ln H_2 – lnH_1)/(t_2–t_1), where t was the time in days and H_1 and H_2 were the plant height at t_1 and t_2 (Hunt, 1982). The total leaf area per plant (TLA, cm^2) was calculated by multiplying the total leaf number per plant and LA (Crescente & *al.*, 2000).

Morphological and Anatomical leaf traits

Measurements of leaf morphological traits were carried out on fully expanded sun leaves (*n*= 50 leaves) collected at the beginning of July from the randomly selected plants. The following parameters were measured: projected fresh leaf surface area (LA, cm^2) excluding petioles (Image Analysis System, DeltaT Devices, UK) and leaf dry mass (DM, mg), drying leaves at 80°C to constant mass. Leaf mass per unit of leaf area (LMA, mg cm^{-2}) was calculated by the ratio of DM and LA. Leaf tissue density (LTD, mg cm^{-3}) was calculated by the ratio of LMA and leaf thickness (L, µm).

Measurements of leaf anatomy were carried out by leaf sections from fully expanded leaves of the selected plants measured by a light microscope. Stomatal density (SD, stomata mm^{-2}) and guard cell length were measured from nail varnish impressions (n= 20 leaves) of the inferior lamina (Galmés & *al.*, 2007) each of them 0.5×1.0 cm, obtained by a Zeiss Axiocam MRc 5 digital camera (Carl Zeiss), with Axiovision AC software. Stomatal area index (SAI) was calculated by the product of mean guard cell length and SD (Ashton & Berlyn, 1994).

Gas Exchange

Gas exchange measurements were carried out in the middle of July (i.e. maximum leaf surface area) according to Gratani & *al.* (2012) and at the beginning of September (*i.e.* beginning of plant senescence) by an infrared gas analyzer (LCPro+, ADC, UK), equipped with a leaf chamber (PLC, Parkinson Leaf Chamber) on fully expanded sun leaves (*n*= 15 per each sampling occasion). Environmental controls within the leaf chamber were maintained to match the outdoor environmental conditions. Net photosynthesis (P_N, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), leaf transpiration (E, mmol $m^{-2}s^{-1}$), photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) and leaf temperature (T_l, °C) were measured from 10:00h to 12:00h under natural conditions on cloud-free days (PPFD ≥ 1600 µmol m^{-2} s^{-1}) to ensure that maximum P_N rates were measured. The instantaneous water use efficiency (WUEi, µmol mol^{-1}) was calculated as the ratio between net photosynthesis and stomatal conductance (Llambí & *al.*, 2003). Leaf respiration in darkness (R_D, µmol m^{-2} s^{-1}) was measured by CO_2 efflux, in each sampling occasion on the same leaves used for P_N measurements. These leaves were shaded during the daylight hours with black paper for 30 min prior to each measurement, to avoid transient posts–illumination bursts of CO_2 (Cai & *al.*, 2005). The ratio between R_D and P_N was calculated.

Microclimate measurements were carried out every 2 hours at ground level during gas exchange measurements. In particular, air temperature (T_a, °C) was measured by a thermo-hygrometer (HD 8901, Delta Ohm, I) and PPFD by a quantum radiometer photometer (LI-189 LI-COR, USA) and a quantum sensor LI-190SA. Moreover, a solar radiation analysis was carried out using tools in ArcGIS Spatial Analyst allowing effects of the sun over a geographic area for specific time periods to be mapped and analyzed. These tools accounted for how daily and seasonal shifts of sun angle variations, orientation and shadows cast by topographic features affect the amount of incoming solar radiation (DPFD, WH m^2).

Biochemical Plant traits

Leaf samples for nitrogen and pigment content were collected on the selected plants in the same occasions of gas exchange measurements. Immediately after collection, leaf samples were kept cool in the dark and transported immediately to the laboratory. Leaf nitrogen content (N_l, mg g^{-1}) was determined by Kjeldahl analysis (n=12). Photosynthetic nitrogen use efficiency (PNUE, µmol g^{-1} s^{-1}) was calculated by the ratio between net photosynthesis and N_l (Poorter & Evans, 1998).

Chlorophyll content (Chl, mg g^{-1}) and carotenoid content (Car, mg g^{-1}) were determined according to Maclachlan & Zalik (1963) and Holm (1954), respectively (n=25). Total Chl content (Chl_{a+b}), Chl/Car and Chl*a*/*b* were calculated.

Statistical Analysis

All statistical tests were performed using statistical software (Statistica, Statsoft, USA). The differences between July and September in physiological and biochemical leaf traits and microclimate measurements were analyzed by one-way ANOVA at $P \leq 0.05$. Results were expressed as mean value±CI 95%. The mean coefficient of variation (CV) was calculated by averaging the coefficient of variation for the selected plant structural (CV_s) and morphological (CV_m), anatomical (CV_a) physiological (CV_p) and biochemical (CV_b) leaf traits. CV was used as measure of phenotypic variability. The relationships between P_N and N_l, g_s and Chl_{a+b} were evaluated by linear regressions. Moreover, correlation coefficients (*R*-Pearson) were determined to test for the relationship between PNUE and plant traits (i.e. H_{max} and RGR_H).

Results

Phenology and Plant traits

Vegetative activity started at the end of May and continued until the middle of July. Flowering started at the middle of June, fruit formation in the first days of July and seed dissemination at the end of August. Plant senescence started at the beginning of September and plants were completely dry in the period between the end of September and the beginning of October. The length of the phenological cycle, from seedling emergence to the end of plant senescence was 128±10 days. The relative growth rate in height (RGR_H) was 0.024±0.004 mm mm^{-1} d^{-1}, the maximum height (H_{max}) 64±4 mm and the total leaf area per plant (TLA) 38±9 cm^2. The coefficient of variation for structural plant traits (CV_s) was 24%.

Morphological and Anatomical leaf traits

Morphological and anatomical leaf traits are show in Table 1. Projected leaf fresh surface area (LA) was 7.4±0.6 cm^2, leaf thickness (L) 94.4±6.3 µm, the leaf mass per unit of leaf area (LMA) 11.8±0.6 mg cm^{-2} and leaf tissue density (LTD) 124.6±14.3 mg cm^{-3}. Stomatal density (SD) was 46.4±1.2 mm^{-2} and stomatal area index (SAI) 2.20±0.05. The coefficient of variation for anatomical (CV_a) and morphological (CV_m) leaf traits were 6% and 15%, respectively.

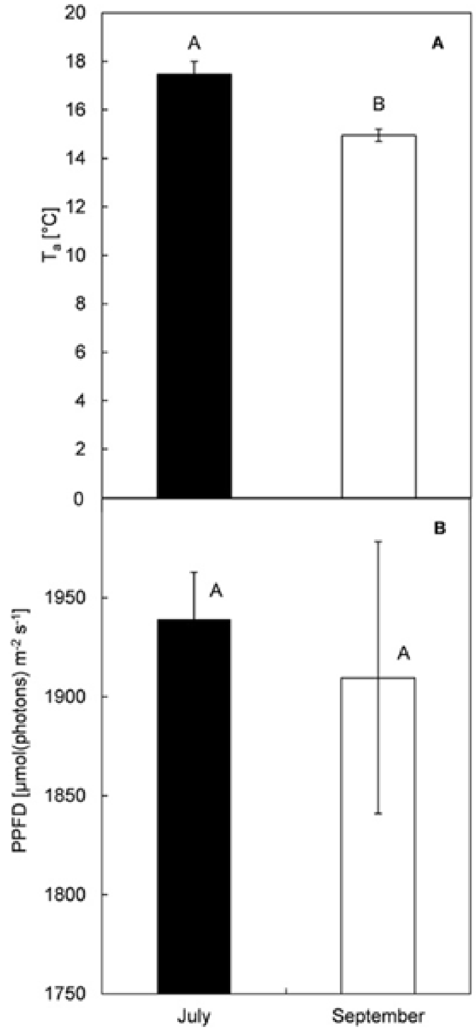
Table 1. Morphological and anatomical leaf traits of *Adonis distorta.* LMA: leaf mass per unit of leaf area, LTD: leaf tissue density, L: leaf thickness; SD: stomatal density; SAI: stomatal area index. Mean value and 95% confidence interval (95% C.I.) are shown. n= 50 for LMA and LTD, n= 20 for L, SD and SAI.

	Mean value	**95% C.I.**
LMA (mg cm $^{-2}$)	11.8	11.2-12.4
LTD (mg cm $^{-3}$)	124.6	110.3-138.9
L (µm)	94.4	88.1-100.7
SD (stomata mm $^{-2}$)	46.4	45.2-47.4
SAI	2.20	2.15-2.25

Gas Exchange

The highest P_N was monitored in July (19.6±2.3µmol m^{-2} s^{-1}) when T_a was 17.47±0.53 °C (Figures 1 and 2) decreasing by 86% in September. g_s had the same P_N trend (0.36±0.03 mol m^{-2} s^{-1} and 0.09±0.04 mol m^{-2} s^{-1}, in July and September; respectively). WUEi was significantly ($P< 0.01$) higher in July (55.1±2.3 µmol $mmol^{-1}$) decreasing by 32% in September. Photosynthetic nitrogen use efficiency (PNUE) was 6.5±0.8 µmol g^{-1} s^{-1} in July decreasing by 59% in September. R_D was 0.70±0.13 µmol m^{-2} s^{-1} in July increasing by more than 100% in September. The ratio R_D/P_N was 0.031±0.002 in July due to the low R_D rates associated to the high P_N rates. It was 0.738±0.200 in September because of the high R_D rates associated to the low P_N rates. The coefficient of variation for physiological traits (CV_p) was 16%.

Figure 1. Air temperature (Ta, A) and photosynthetic photon flux density (PPFD, B) measured in July and September. The mean values (± 95% confidence interval) are shown (n=15). Mean values with the same letters are not significantly different (ANOVA; p> 0.05).



Biochemical Leaf traits

Leaf nitrogen content (N_l), total chorophyll content (Chl_{a+b}), carotenoid content (Car) and the ratio between chlorophyll and carotenoid content (Chl/Car) are shown in Table 2. N_l was significantly ($P < 0.01$) higher in July (25.9±1.3 mg g^{-1}) decreasing by 57% in September. Chl_{a+b} had the same N_l trend with significantly ($P < 0.01$) higher content in July (0.88±0.13 mg g^{-1}) decreasing by 90% in September. In July Chl*a* and Chl*b* were 0.66±0.10 mg g^{-1} and 0.28±0.14 mg g^{-1}, respectively. Chl*a* and Chl*b* decreased by 95% and 98%, respectively, in September. The Chl/Car ratio was 4.19±0.75 in July decreasing by 83% in September due to the greater Chl decrease in respect to the Car decrease (62%). The coefficient of variation for biochemical (CV_b) leaf traits was 19%. The results of linear regressions highlighted significant relationships ($P < 0.01$) between P_N and N_l (R^2= 0.96), P_N and g_s (R^2= 0.86) and P_N and Chl_{a+b} (R^2= 0.83) (Figure 2). Moreover, PNUE was significantly correlated with both H_{max} (R= –0.23) and RGR_H (R= –0.48; Figure 3).

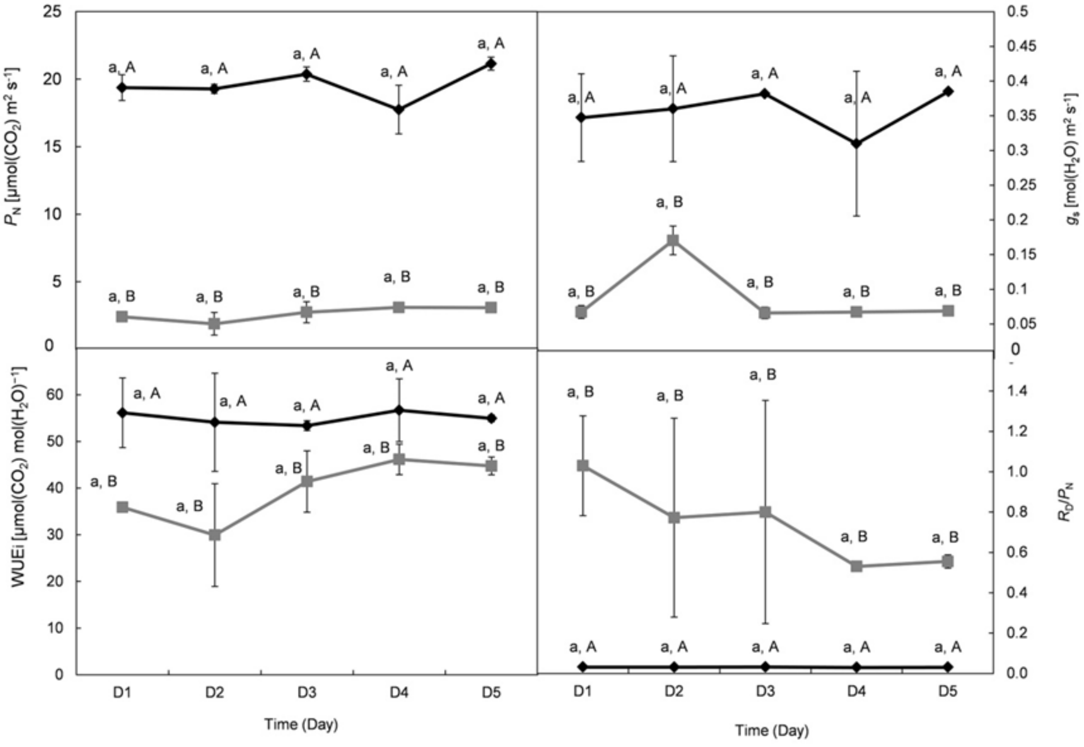
Discussion

High-mountain environments are characterized by several features which can determine contrasting environmental conditions such as low atmospheric pressure, low air temperature, high irradiance, strong wind action, diurnal environmental fluctuations, and unpredictable limitations of soil water and nutrients availability (Larcher & *al.*, 2010). Our results on the whole highlight the adaptive strategy of *A. distorta* at the highest altitude of its distribution area. The

Table 2. Pigment content of *Adonis distorta* leaves in July (i.e. full vegetative activity) and September (i.e. at the beginning of the senescence phase). Chl_{a+b}: chlorophyll a+b; Chla/b: ratio of chlorophyll a to chlorophyll b content; N: total leaf nitrogen content; Car: carotenoid content; Chl/Car: ratio of chlorophyll to carotenoid content. Means with different letters between July and September are significantly different (Repeated measure ANOVA, p<0.05). 95% confidence interval (95% C.I.) are shown. n=12 for N measurements; n=25 for the others variables.

	July		**September**	
	Mean value	**95% C.I.**	**Mean value**	**95% C.I.**
Chl_{a+b} (mg g^{-1})	0.88a	0.75-1.01	0.09b	0.04-0.14
Chla/b	2.95a	2.61-3.29	6.56b	6.16-6.96
N (mg g^{-1})	25.9a	24.5-27.1	11.05b	9.80-12.3
Car (mg g^{-1})	0.21a	0.20-0.22	0.08b	0.07-0.09
Chl/Car	4.19a	3.44-4.94	0.69b	0.48-0.90

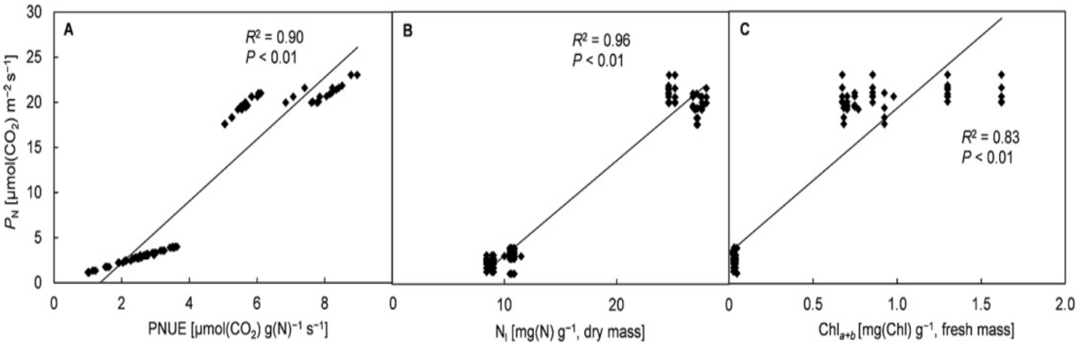
Figure 2. Trends of net photosynthetic rates (P_N) (A), stomatal conductance (gs) (B), instantaneous water use efficiency (WUEi) (C) and ratio between respiration rate (R_D) and net photosynthetic rate (R_D/P_N) (D) of *Adonis distorta* measured at the middle of July (*black line*) and at the beginning of September (*dotted line*). The mean values (± 95% confidence interval) are shown. Each point on the graph represents daily mean measurements (*n*= 15 leaves). Lowercase letters show significant intra-month differences, capital letters show significant inter-month differences. Mean values with the same letters are not significantly different (ANOVA; $P > 0.05$).



area is characterized by an extremely cold climate with a mean annual temperature of 7.6±6.5°C and a snow cover for 7–8 months during the year. The scree soil has a low mineral content as attested by a high C/N (9.98±0.33) and a low SOM (2.55±0.28%). In response to such conditions, *A. distorta* completes the phenological cycle in 128±10 days, starting immediately after the snowmelt at the end of May. Plant size in mountain environments is related to the roughness of the ground surface and wind speed (Whitehead, 1951). Thus, the highest RGR_H two weeks after the beginning of the *A. distorta* vegetative activity allows the achievement of the definitive height (64±4 mm) in 43±11 days. The small *A. distorta* size may be advantageous during the vegetative activity in terms of limiting the effects of low temperature and strong wind action (Taschler & Neuner, 2004), and in summer of overheating and desiccation mainly when low precipitation is associated to an excess of radiation (Körner, 2003) (DPFD= 1.94 e^{14}±3.19 e^{13} WH m^{-2}). Moreover, the high *A. distorta* LMA (11.8±0.6 mg cm^{-2}), compared to other herbaceous species (Wright & *al.*, 2004), associated to a relatively high LTD (124.6±14.3 mg cm^{-3}), seem to be an adaptive trait in reducing leaf water loss by transpiration (Puglielli & *al.*, 2015) and providing a photo-protection function (Niinemets, 2001). At the same time, Poorter & *al.* (2009) highlight that species with high LMA have advantage under adverse growing conditions where slow tissue turnover is favored.

The photosynthetic performance is one of the most critical aspects of plant response to environmental changes in mountain areas (Shen & *al.*, 2008). The high *A. distorta* P_N and Chl_{a+b} in July are justified by the favorable air temperature in the range 16.5°C−18.4°C.

Figure 3. Regression analysis between: net photosynthetic rates (P_N) and leaf nitrogen content (N_l, A), net photosynthetic rates (P_N) and total chlorophyll content (Chl_{a+b}, B), for *Adonis distorta.* Mean individual daily measurements carried out in July and September were used as experimental units (n=50, $P \leq 0.01$).



P_N decreased by 87% in September at the beginning of plant senescence when air temperature is 14.9±0.42°C. The P_N decrease in this period is justified by the fact that carbon assimilation is replaced by catabolism of chlorophyll and macromolecules (i.e. proteins) (Lim & *al.*, 2007). This results in a higher decrease of both Chl_{a+b} (by 90%) and N_l (by 57%) during plant senescence. Chlorophyll breakdown during plant senescence proceeds throughout the conversion of Chl*b* to Chl*a* before entering the catabolic pathway (Pružinská & *al.*, 2005), resulting in a more than 100% Chl*a*/*b* increase. The Chl_{a+b} breakdown is a multistep pathway that, in addition to the required catabolic enzymes, requires transport processes for the export of catabolites (Matile & *al.*, 1988). This justifies the highest R in September which results in a higher R/P_N, according to the results of Gratani & *al.* (2012). The g_s follows the same P_N trend, as attested by the significant correlation between the two variables. The monitored *A. distorta* P_N and g_s are higher compared to other mountain species growing at lower altitudes (Woodward, 1986) confirming the general trend for which plants tend to maximize net photosynthesis and stomatal conductance with increasing altitude in relation to the shorter growing season (Gornall & Guy, 2007). Moreover, the low SD (46.6±7.5 mm^{-2}), in the range of other mountain species (Woodward, 1986), improves WUEi during the growing season (Woodward, 1987). At high altitudes, where the growing season is short, P_N is maximized through a higher g_s and the nitrogen economy of the leaves (*i.e.* a higher N_l) (Woodward, 1986). This is attested in *A. distorta* by the significant relationships between P_N and N_l. Wielgolaski & *al.* (1975) highlight that the general trend of soil N content in altitude is opposite to that observed for leaves due to a slower nutrient cycling and, thus, a low nitrogen availability (Gornall & Guy, 2007). Moreover, a more efficient utilization of the available N_l for dry-matter production is related to a decreased plant size (Körner & *al.*, 1989). The observed negative correlations between PNUE and plant height and between PNUE and RGR_H confirm this trend in *A. distorta*. In addition the P_N and R_D variations determine a positive carbon balance during the growing season becoming indicative of *A. distorta* carbon use efficiency (Loveys & *al.*, 2002).

Climate change could be a selective factor leading to the adaptive evolution of key plant traits (Valladares & *al.*, 2007). As climatic conditions change, the frequency of certain plant traits is expected to change (Pellissier & *al.*, 2010). Functional trait variability at the intra-specific level is usually assumed to have an adaptive significance (Scheepens & *al.*, 2010). In the context of a rapid climate change, phenotypic plasticity can be a crucial determinant of plant response, both in short-and long term (Nicotra & *al.*, 2010). On the whole, the measured CV (16%) reflects the *A. distorta* intra-population plant and leaf trait variability (*i.e.* the phenotypic plasticity).

In particular, the highest phenotypic plasticity is measured for plant traits (CV_s=24%) than for biochemical (CV_b=19%) physiological (CV_p=16%), morphological (CV_m=15%) and anatomical leaf traits (CV_a=6%). Thus, the highest value for CV_s confirms the importance for *A. distorta* in modifying its plant traits in order to maximize the utilization of available soil nitrogen which could be the result of its patchy presence in high mountain soils (Whitehead, 1951). Moreover, the greater physiological plasticity in respect to morphological and anatomical plasticity confirms the general trend of the importance of physiology in plant acclimation to adverse environmental conditions (Gratani & *al.*, 2014).

Comparative studies on the adaptive strategies among other *A. distorta* populations on the Central Apennines could give knowledge of the species response to environmental heterogeneity within the species distribution area. Moreover, the results could be an important tool for this endemic species conservation programmes considering that to date there is no protocol for germination (Frattaroli & *al.*, 2013) which could be used for reinforcement projects of wild populations. Knowing the potential growth of Red-listed species and their resource-use capability facilitates efficient allocation of management efforts to the most critical areas with high conservation value (Crescente & Gratani, 2013).

References

Ashton, P.M.S. & Berlyn, G.P. 1994. A comparison of leaf physiology and anatomy of Quercus (section Erythrobalanus – Fagaceae) species in different light environments. Am. J. Bot. 81: 589-597.

Cai, Z.-Q., Slot, M. & Fan, Z.-X. 2005. Leaf development and photosynthetic properties of three tropical tree species with delayed greening. Photosynthetica 43: 91-98.

Crescente, M.F. & Gratani, L. 2013. Differences in morphological, physiological and growth traits between two endemic subspecies of Brassica rupestris Raf.: implications for their conservation. Am. J. Plant Sci. 4: 42-50.

Crescente, M.F., Gratani, L. & Larcher, W. 2000. Shoot growth efficiency and production of Quercus ilex L. in different climate. Flora 197: 2-9.

Di Pietro, R., Pelino, G., Stanisci, A. & Blasi C. 2008. Phytosociological features of Adonis distorta and Trifolium noricum subsp. praetutianum, two endemics of the Apennines (peninsular Italy). Acta Bot. Croat. 67 (2): 175-200.

Dirnböck, T., Essl, F. & Rabitsch, W. 2011. Disproportional risk for habitat loss of high-altitude endemic species under climate change. Glob. Change Biol. 17: 990-996.

Engler R., Randin, C., Thuiller, W., Dullinger, S., Zimmerman, N.E., […] & Guisan, A. 2011. 21st century climate change threatens mountain flora unequally across Europe. Glob. Change Biol. 17: 2330-2341.

Frattaroli, A.R., Di Martino, L. & Di Cecco, V., Catoni, R., Varone, L., Di Santo, M. & Gratani, L. 2013. Seed germination capability of four endemic species in the Central Apennines in Italy: relationship between seed size and germination capability. Lazaroa 34: 43-53.

Galmés, J., Flexas J. & Savé, R. & Medrano, H. 2007. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant Soil 290: 139-155.

Giorgi, F. & Lionello, P. 2008. Climate change projections for the Mediterranean region. Global Planet. Change 63: 90-104.

Gornall, J.L. & Guy, R.D. 2007. Geographic variation in ecophysiological traits of black cottonwood (Populus trichocarpa). Can. J. Bot. 85: 1202-1213.

Gratani L., Catoni, R. & Pirone, G., Fratarolli, A.R. & Varone, L. 2012. Physiological and morphological leaf trait variations in two Apennine plant species in response to different altitudes. Photosynthetica 50: 15-23.

Gratani, L., Crescente, M.F. & D'Amato, V., Ricotta, C., Frattaroli, A.R. & Puglielli, G. 2014. Leaf traits variation in Sesleria nitida growing at different altitudes in the Central Apennines. Photosynthetica 52(3): 386-396.

Holm, G. 1954. Chlorophyll mutations in barley. Acta Agr. Scand. 4: 457-471.

Hunt, R. 1982. Plant Growth Curves. The Functional Approach to Growth Analysis. Edward Arnold, London.

Körner, C. 1999. Alpine Plant Life. Springer Verlag, Berlin.

Körner, C., Neumayer, M., Pelaez-Menendez-Rieldl, S. & Smeets-Scheel, A. 1989. Functional morphology of mountain plants. Flora 182: 353-383.

Körner, C. 2003. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems, 2nd ed. Springer, Berlin.

Larcher, W., Kainmüller, C. & Wagner, J. 2010. Survival types of high mountain plants under extreme temperatures. Flora 205: 3-18.

Llambí, L., Fontaine, M., Rada, F., Saugier, B. & Sarmiento, L. 2003. Ecophysiology of dominant plant species during old-field succession in a high tropical Andean ecosystem. Arct. Antarct. Alp. Res. 35: 447-453.

Lim, P.O., Kim, H.J. & Nam, H.G. 2007. Leaf senescence. Ann. Rev. Plant Biol. 58: 115-136.

Loveys, B.R., Scheurwater, I., Pons, T.L., Fitter, A.H. & Atkin, O.K. 2002. Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast-and slow-growing plant species. Plant Cell Environ. 25: 975-987.

Maclachlan, S. & Zalik, S. 1963. Plastid structure, chlorophyll concentration, and free aminoacid composition of a chlorophyll mutant of barley. Can. J. Bot. 41: 1053-1062.

Martín-Bravo S., Valcárcel, V. & Vargas, P. & Luceño, M. 2010. Geographical speciation related to Pleistocene range shifts in the western Mediterranean mountains. Taxon 59: 466-482.

Matile P., Ginsburg, S. & Schellenberg, M. & Thomas, H. 1988. Catabolites of chlorophyll in senescing barley leaves are localized in the vacuoles of mesophyllcells. P. Natl. Acad. Sci. USA 85: 9529-9532.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F. & van Kleunen, M. 2010. Plant phenotypic plasticity in a changing climate. Trends Plant Sci. 15: 684-692.

Niinemets, Ü. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology 82(2): 453-469.

Pellissier, L., Bråthen, K.A., Pottier, J., Randin, .C.F., Vittoz, P., Dubuis, A., Yoccoz, N.G., Alm, T., Zimmermann, N.E. & Guisan, A. 2010. Species distribution models reveal apparent competitive and facilitative effects of a dominant species on the distribution of tundra plants. Ecography 33: 1004-1014.

Poorter, H. & Evans, J.R. 1998. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116: 26-37.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. & Villar, R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182: 565-588.

Pružinská, A., Tanner, G., Aubry, S., Anders, I., Moser, S., Müller, T., Ongania, K.H., Kräutler, B., Youn, J.Y., Liljegren, S.J. & Hörtensteiner, S. 2005. Chlorophyll breakdown in senescent Arabidopsis leaves. Characterization of chlorophyll catabolites and of chlorophyll catabolic enzymes involved in the degreening reaction. Plant Physiol. 139: 52-63.

Puglielli, G., Crescente, M.F., Frattaroli, A.R. & Gratani, L. 2015. Leaf mass per area (LMA) as a possible predictor of adaptive strategies for two species of Sesleria (Poaceae): analysis of morphological, anatomical and physiological leaf traits. Ann. Bot. Fenn. 52: 135-143.

Sanz-Elorza, M., Dana, E.D. & Gonzalez, A. 2003. Changes in the high-mountain vegetation of the central Iberian Peninsula as a probable sign of global warming. Ann. Bot. 92: 1-8.

Scheepens, J.F., Frei, E.S. & Stöcklin, J. 2010. Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. Oecologia 164: 141-150.

Shen, H., Tang, Y., Muraoka, H. & Washitani, I. 2008. Characteristics of leaf photosynthesis and simulated individual carbon budget in Primula nutans under contrasting light and temperature conditions. J. Plant Res. 121:191-200.

Taschler, D. & Neuner, G. 2004. Summer frost resistance and freezing patterns measured in situ in leaves of major alpine plant growth forms in relation to their upper distribution boundary. Plant Cell Environ. 27: 737-746.

Thuiller, W., Lavorel, S., Araujo, M.B., Sykes, M. & Prentice, C. 2005. Climate change threats to plant diversity in Europe. P. Natl. Acad. Sci. USA. 102: 8245-8250.

Valladares F., Gianoli, E. & Gómez, J.M. 2007. Ecological limits to plant phenotypic plasticity. New Phytol. 176: 749-763.

van Gils H., Conti, F., Ciaschetti, G. & Vestinga, E. 2012. Fine resolution distribution modelling of endemics in Majella National Park, Central Italy. Plant Biosyst. 146: 276-287.

Wielgolaski, F.E., Kjevik, S. & Kallio, P. 1975. Mineral content of tundra and forest tundra plants in Fennoscandia. In: Wielgolaski, F.E. (Ed.). Fennoscandian tundra ecosystems. Part 1. Plants and microorganisms. Pp. 316-332. Springer-Verlag, Berlin.

Whitehead, F.H. 1951. Ecology of the altopiano of Monte Maiella, Italy. J. Ecol. 39(2): 330-355.

Woodward, F.I. 1986. Ecophysiological studies on the shrub Vaccinium myrtillus L. taken from a wide altitudinal range. Oecologia 70: 580-586.

Woodward, F.I. 1987. Stomatal numbers are sensitive to increase in CO_2 from pre-industrial levels. Nature 327: 617-618.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D., Baruch, Z, […] & Villar, R. 2004. The worldwide leaf economics spectrum. Nature 428: 821-827.