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Physiological and morphological leaf trait variations in two Apennine plant species in response to different altitudes

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Abstract

Morphological and physiological traits of *Crepis pygmaea* L. subsp. *pygmaea* and *Isatis apennina* Ten. ex Grande growing at different altitudes in the Gran Sasso Massif (Abruzzo, Italy) were analyzed. The two populations of *C. pygmaea* and *I. apennina* growing at the highest altitude (C_{p2} and I_{p2} at 2,310 m a.s.l. and 2,350 m a.s.l., respectively) had a lower leaf mass area (LMA) than the two populations growing at the lowest altitude (C_{p1} and I_{p1} at 2,250 m a.s.l. and 2,310 m a.s.l., respectively). Leaf tissue density (LTD) had the same LMA trend, decreasing 23 and 10% in *C. pygmaea* and *I. apennina*, respectively, from the highest to the lowest altitude. *C. pygmaea* and *I. apennina* had the highest photosynthetic rates (P_N) in July decreasing on an average 17 and 30%, respectively, in August and 50 and 38%, respectively, in September. Leaf respiration (R) in I_{p1} and I_{p2} had the same trend as C_{p1} and $C_{p2,}$ showing the highest rates in September. Global warming could drive *C. pygmaea* and *I. apennina* toward higher altitudes in the Gran Sasso Massif. Nevertheless, *C. pygmaea* with the higher plasticity index (PI) both at physiological and at morphological levels (0.50 and 0.35, respectively) might have a competitive advantage over *I. apennina* over the long term.

Additional key words: gas exchange; leaf mass per unit of leaf area; mountain species; plasticity index.

Introduction

The impact of global warming on terrestrial ecosystems has been shown to be greater in arctic tundra and high mountain regions than in low latitude areas (Larcher *et al.* 2010). In particular, the projected rate of warming in the mountain ecosystems is expected to be up to three times higher than the global average rate of warming recorded during the 20th century (Nogués-Bravo *et al.* 2007). A recent bioclimatic envelope study on 1,350 European plant species underlines that species from mountain areas might be disproportionably sensitive to climate change and southern mountain regions in Europe are predicted to experience particularly severe species losses (Lenoir *et al.* 2010). Dirnböck *et al.* (2011) hypothesize a rapid worldwide increase in plant species extinction risk. Moreover, species-specific reduction in fitness and diversity could change community dynamics by altering species-competitive abilities. The combination of reduced fitness interacting with a potential reduction in diversity may lead to local or widespread population extinction (Jump and Peñuelas 2005).

One of the predicted consequences of global climate change is the movement of plant species to higher elevations and latitudes as the climate to which they are adapted is displaced (Jump and Peñuelas 2005). An increasing number of studies show altitudinal range shifts for mountain plant species, in particular for alpine ecosystems (Erschbamer *et al.* 2009). Moreover, a reduction of the snow cover could expose plants, which are currently protected by snow in winter and spring, to greater extremes of temperature with an increased risk of damage by frost, desiccation, and insolation (Neuner *et al.* 1999). A drastic decrease of the distribution area, or even extinction of plant species, can be the consequence of migration processes towards higher altitudes; rates and patterns of these dynamics will be highly dependent on the habitat preference of a particular species and on its

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Abbreviations: Chl – chlorophyll content; DM – leaf dry mass; E – transpiration rate; g_s – stomatal conductance; ; H – plant height; L – leaf thickness; LA – leaf area; LMA – leaf mass per unit of leaf area; LTD – leaf tissue density; P_g – gross photosynthetic rate; PI – plasticity index; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; PCA – Principal component analysis; R – leaf respiration rate; RH – relative air humidity; RWC_{pd} – predawn relative leaf water content; T_a – air temperature; TLA – total leaf area per plants; WUE – instantaneous water-use efficiency; Ψ_{pd} – predawn leaf water potential.

functional key traits (Pauli *et al.* 2003). However, habitat fragmentation is likely to impede migration in the future (Etterson and Shaw 2001).

Populations may persist in their current areas and withstand environmental changes if they have adaptive capacity (Lindner *et al.* 2009). Recent studies indicate substantial adaptive potential as reflected by high heritability estimates for traits likely to be selected (Savolainen *et al.* 2004). When environments within the range of a species differ, it may be unlikely that any single phenotype confers high fitness in all situations, and phenotypic plasticity can provide increased phenotypic tolerance. In particular, phenotypic plasticity will determine the short-term ecological response of a species to climate change and, in some cases, could directly buffer the effects of such change (Theurillat and Guisan 2001).

Under rapid climate change phenotypic plasticity rather than genetic diversity is likely to play a crucial role in allowing plant species to persist in their environments (Vitasse *et al.* 2010). The current rate of global warming might be too rapid for natural migration to successfully deliver species toward suitable habitats (Vitasse *et al.* 2010). Moreover, in alpine areas around the world, it is expected that warming will allow invasion from lower elevations creating pressure for upward migration of alpine species (Jump and Peñuelas 2005, Byars *et al.* 2007). Nevertheless, the physiological response of plant species growing in mountain areas to the hypothesized increase of air temperature has been scarcely understood. High mountain vegetation can be used as a sensitive "ecological indicator" of climate change effects, because of its low biotic complexity, and thus abiotic factors, particularly climate, dominate over biotic ones, such as competition (Pauli *et al.* 2003).

Thus, considering that mountain ecosystems are centers of endemism and important areas for biodiversity conservation (Nogués-Bravo *et al.* 2007), knowledge of life history traits of endemic and threatened species is important to assess their response to global warming, in particular, when wild populations number and size are small (Gratani *et al.* 2011). A significant threat to small and isolated populations is their sensitivity to environmental, genetic and demographic stochasticity (Morgan 2000). Moreover, there is little information on the adaptive potential in environments that are particularly threatened by climate change such as high altitude mountain areas.

Crepis pygmaea L. subsp. *pygmaea* and *Isatis apennina* Ten. ex Grande are two perennial herbaceous rhizomatous species (Pignatti 1982) distributed in South Europe. *C. pygmaea* and *I. apennina* colonize high mountain areas from the North to the Centre of Italy. These species are included in the Red List of the Italian Flora, as low risk (LR) species for Abruzzo (Conti *et al.* 1997, Rossi *et al.* 2008), where they grow exclusively on slopes with unstable limestone screes (Di Pietro *et al.* 2001).

The aim of this research was to analyze morphological and physiological leaf traits of two populations of *C. pygmaea* subsp. *pygmaea* and *I. apennina*, respectively, growing at different altitudes in the Gran Sasso Massif. We addressed the question of whether altitudinal variations in their morphological and physiological traits could be indicative of their future adaptive potential to survive global warming. Also we investigated whether the selection of functional key traits might be important for monitoring *C. pygmaea* and *I. apennina* wild populations over the long term.

Materials and methods

The study sites were located in the Gran Sasso Massif. The Gran Sasso Massif consists mainly of carbonate rocks (Giraudi and Frezzotti 1997), and its vegetation is primary and secondary strep grassland up to 2,100–2,200 m a.s.l. (a pioneer vegetation on mobile and stabilized screen soils) and then alpine tundra from 2,300 m a.s.l. to the peak (Giglio and Tammaro 1996).

The climate of the study area was analyzed using data from the nearest Meteorological Station to the study sites (Campo Imperatore at 1,950 m a.s.l., 42°26'24"N; 13°34'16"E; data were available for the period 1960 to 1990, and the year 2010). The average minimum air temperature of the coldest month (January) was –6.1 ± 2.1°C, and the average maximum air temperature of the hottest months (July and August) was 14.2 ± 1.6°C (mean of the period 1960 to 1990). Snow covered the area from October to May. Moreover, Baldoni *et al.* (1999) attested that the air temperature decreased by 0.58°C every 100 m of altitude for the southern slope and 0.62°C for the northern slope of the Gran Sasso massif. During the study year (2010) the average minimum air temperature of the coldest month (December) was –8.1 ± 5.6°C and the average maximum air temperature of the hottest month (July) was 17.0 ± 3.9°C.

Two wild populations of each species (*C. pygmaea* and *I. apennina*), growing at different altitudes, were analyzed. The two populations of *C. pygmaea* (C_{p1} and C_{p2}) developed on calcareous screes: C_{p1} at 2,250 m a.s.l. (42°27'58''N; 13°33'50''E) and C_{p2} at 2,310 m a.s.l. (42° 27'60"N; 13° 27'55"E). C_{p1} was characterized by a population density of 3 individuals per m^2 and C_{p2} by 2 individuals per m^2. The two populations of *I. apennina* (I_{p1} and I_{p2}) developed on stony soils and screes: I_{p1}–at 2,310 m a.s.l. (42° 27'60"N; 13° 27'55"E), and I_{p2} at 2,350 m a.s.l. (42°27'48''N; 13° 27' 50''E). I_{p1} was characterized by a population density of 5 individuals per m^2 and I_{p2} by 7 individuals per m^2. Sampling areas 10 × 10 m^2 each (four areas per each of the considered populations) were established in June 2010 at the beginning of the growing season.

Experiments were carried out from the beginning of June to the beginning of September 2010, when the snow did not cover the study areas, on ten representative plants randomly selected from each of the two populations of both *C. pygmaea* and *I. apennina*. Physiological measurements started in the middle of July when the leaves were fully expanded. In the middle of August both the species were in the flowering phase followed by seed formation at the end of August and then by the senescence phase in the middle of September.

Microclimatic measurements: In each sample area and during each day of measurement, air temperature (T_a) and relative air humidity (RH) were measured with a portable thermo-hygrometer (*HD 8901, Delta Ohm*, Italy), at 20 cm above the ground, from 8.00 to 15.00 h, every 30 min. The results of T_a and RH measurements are shown in Fig. 1.

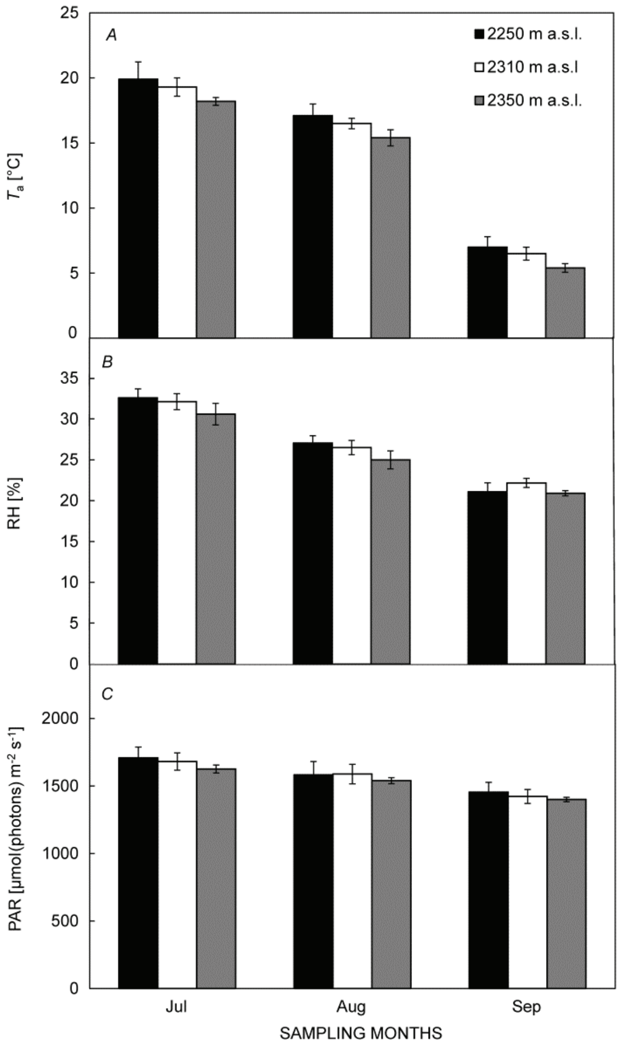

Fig. 1. Trend of *A*: Air temperature (T_a), *B*: relative air humidity (RH) and *C*: photosynthetically active radiation (PAR) monitored during the study period at the different altitude where populations of *C. pygmaea* and *I. apennina* were sampled.

Plant traits including plant height (H) and total leaf area per plant (TLA), were measured on the plants (ten plants per population) at the end of the vegetative activity (beginning of July).

H was defined as the maximum vertical distance from the soil level to the highest point of each plant. TLA was calculated by multiplying the total leaf number per plant and the mean leaf area per plant.

Leaf morphology: Measurements of leaf morphology included leaf area (LA), obtained by the *Image Analysis System* (*Delta-T Devices*, UK), leaf dry mass (DM) when oven-dried at 80°C to constant mass. Leaf thickness (L) was measured on leaf sections from fresh, fully expanded leaves, and measured by light microscope. Leaf mass per unit of leaf area (LMA) was calculated by the ratio of leaf DM and LA. Leaf tissue density (LTD) was calculated by the ratio of LMA and total leaf thickness. Measurements were carried out on two fully expanded leaves from each plant (*n* = 20 leaves per population) collected at the beginning of July.

Gas-exchange measurements were carried out by an infrared gas analyser (*LCA-Pro*, *ADC*, UK), equipped with a leaf chamber (*PLC, Parkinson Leaf Chamber*, UK).

Measurements were made in July (14th–16th), August (20th–22th), and September (10th–12th). Sampling days were characterised by comparable climatic conditions. Net photosynthetic rates (P_N), stomatal conductance (g_s), transpiration rates (E), leaf respiration rate (R), and PAR were carried out under natural conditions, on cloud-free days, in the morning, from 8.00 to 12.00 h, to ensure that maximum P_N were measured. During gas-exchange measurements CO_2 concentration inside the leaf chamber was set at 375 ppm and PAR was ≥ 1,400 µmol(photon) m^{-2} s^{-1} (saturating level for high mountain plants, Shen *et al.* 2008). Environmental controls within the leaf chamber were maintained to match the outdoor environmental conditions (Ow *et al.* 2008).

The instantaneous water-use efficiency (WUE) was calculated as the ratio between P_N and E. On each sampling occasion, R measurements were carried out after P_N ones (on the same leaves), as CO_2 efflux, darkening leaf chamber by a black paper, according to Cai *et al.* (2005), for 30 min prior to each measurement, to avoid transient post-illumination bursts of CO_2 releasing (Atkin *et al.* 1998a,b). On each sampling day, measurements were carried out on two fully expanded leaves from each selected plant (*n* = 20 leaves per population). The R and P_N plotted data represented the mean of the maximum measured rates of the three measurement days per month.

Gross photosynthesis (P_g) was calculated, for each sampling occasion during the study period, from P_N plus R.

Chlorophyll content (Chl) was measured using a *SPAD-502* meter (*Konica Minolta Sensing*, *Inc.,* Osaka, Japan).

Chl measurements were carried out on the same leaves used for gas-exchange measurements ($n = 20$ leaves per population), after cleaning the surface dust from each leaf, and on various points on the surface of each leaf sample.

Water relations: Predawn leaf water potential (Ψ_{pd}) was measured on two fully expanded leaves per plant ($n = 20$ leaves per population) with a pressure chamber (*SKPM 1400 Skye Instruments,* UK). Measurements were carried out at the same time as gas-exchange measurements on each sampling occasion. Relative leaf water content at predawn (RWC_{pd}) was calculated on the same leaves used for Ψ_{pd} measurements, as: RWC = (FM – DM)/(TM – DM) × 100, where FM was the leaf fresh mass, DM the leaf mass after drying at 90°C until constant mass was reached and TM the leaf mass after rehydration until saturation for 48 h at 5°C in the darkness.

Data analysis and plasticity index: All statistical tests were performed using a statistical software package (*Statistica, Statsoft*, USA). Differences in leaf traits were determined by analysis of variance (*ANOVA*), and *Tukey* test for multiple comparisons. All data shown were the means ± SE. Differences were considered significant at $p \leq 0.05$. The mean index of phenotypic plasticity (PI) at population level was calculated for each variable as the difference between the minimum and the maximum mean values divided by the maximum mean value (Valladares *et al.* 2000). At species level, the phenotypic plasticity index was calculated by averaging PI obtained for each considered leaf variable. PI scaled from 0 to 1 at population and species level. Principal component analysis (PCA) was carried out in order to evaluate the colinearity among the leaf variables, in both the two populations of *C. pygmaea* and *I. apennina*, respectively. The analysis was performed on a standardized matrix. The matrix was subjected to a rotated principal component analysis with the objective of summarising the main factors determining the variation of the analysed leaf traits.

Results

Plant traits: H was not significantly different between C_{p1} and C_{p2} (5.3 ± 0.6 cm, mean value) and between I_{p1} and I_{p2} (3.0 ± 0.5 cm, mean value). TLA was not significantly different between C_{p1} and C_{p2} (102 ± 10 cm^2, mean value), and between I_{p1} and I_{p2} (413 ± 15 cm^2, mean value).

Leaf morphological traits: LA was significantly different between C_{p1} (7.7 ± 0.1 cm^2) and C_{p2} (6.1 ± 0.3 cm^2) and between I_{p1} (9.0 ± 0.2 cm^2) and I_{p2}(6.8 ± 0.1 cm^2) (Table 1).

LMA of C_{p2} was significantly lower (21%) than the value for C_{p1}, and LMA of I_{p2} was significantly lower (9%) than that of I_{p1}. LTD of C_{p2} was significantly lower (23%) than C_{p1} and LTD of I_{p2} was significantly lower (20%) than I_{p1}.

Gas exchange: The two *C. pygmaea* populations had the same P_N trend during the study period (Fig. 2*A*). No significant difference between P_N of C_{p1} and C_{p2} was found in July (T_a = 19.3–19.9°C), when both the populations had the highest P_N [16.4 ± 0.4 μmol(CO$_2$) m^{-2} s^{-1}, mean value of C_{p1} and C_{p2}]. In August (T_a = 16.5–17.1°C), P_N significantly decreased by 24 and 9% in C_{p1} and $C_{p2,}$ respectively, compared to the maximum. In September (T_a = 6.5–7.0°C), P_N decreased by 56 and 44% compared to the maximum in C_{p1} and C_{p2}, respectively. g_s had the same trend as P_N in C_{p1} and C_{p2} reaching the highest rates in July [0.30 ± 0.01 and 0.26 ± 0.02 mol(H$_2$O) m^{-2} s^{-1}, respectively] and decreasing by 53 and 31%, respectively, in September (Fig. 2*B*). In September C_{p1} had a 19 % lower WUE than C_{p2} (Fig. 2*C*).

The highest P_N of *I. apennina* was monitored in July (T_a = 18.2–19.3°C), and I_{p2} had a significantly higher P_N (by 8%) than I_{p1} (Fig. 2*A*). Compared to the maximum, P_N significantly decreased by 32 and 27%, in I_{p1} and I_{p2},

Table 1. Morphological leaf traits at full leaf expansion and total leaf area per plant of the populations of *C. pygmaea* ($C_{p1}$and C_{p2} growing at 2,250 m a.s.l and at 2,310 m a.s.l, respectively) and *I. apennina* (I_{p1} and I_{p2} growing at 2,310 m a.s.l and at 2,350 m a.s.l., respectively). DM – leaf dry mass; LA – projected leaf surface area; LMA – leaf mass per unit leaf area; LTD – leaf tissue density; TLA – total leaf area per plant. Mean values (± SE) are shown ($n = 20$ for morphological leaf traits and $n = 10$ for TLA). Within each column different *capital letters* indicate significant differences between C_{p1} and C_{p2} while different *lowercase letters* indicate significant differences between I_{p1} and I_{p2} (*Tukey* test, $p \leq 0.05$).

Population	DM [mg]	LA [cm^2]	LMA [mg cm^{-2}]	LTD [mg cm^{-3}]	TLA [cm^2]
C_{p1}	55.9 ± 1.4^A	7.7 ± 0.1^A	7.3 ± 0.2^A	155 ± 4^A	112 ± 10^A
C_{p2}	35.9 ± 1.7^B	6.1 ± 0.3^B	5.8 ± 0.1^B	120 ± 7^B	92 ± 8^A
I_{p1}	76.9 ± 1.7^a	9.0 ± 0.2^a	8.6 ± 0.2^a	168 ± 5^a	428 ± 16^a
I_{p2}	52.3 ± 1.5^b	6.8 ± 0.1^b	7.8 ± 0.1^b	135 ± 3^b	398 ± 12^a

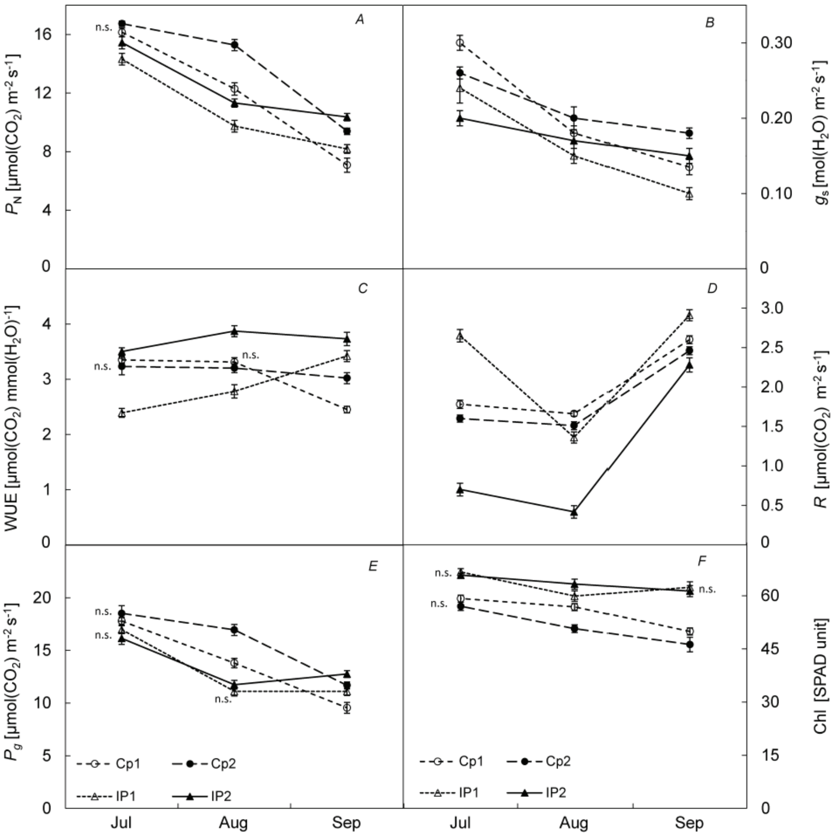

Fig. 2. Trend of *A*: net photosynthetic rate (P_N), *B*: stomatal conductance (g_s,), *C*: instantaneous water use efficiency (WUE), *D*: leaf respiration (*R*), *E*: gross photosynthetic rate (P_g), and *F*: chlorophyll (Chl) content of *C. pygmaea* populations (C_{p1} and C_{p2} growing at 2,250 m a.s.l and at 2,310 m a.s.l, respectively) and *I. apennina* populations (I_{p1} and I_{p2} growing at 2,310 m a.s.l and at 2,350 m a.s.l, respectively) during the study period. The mean value for each month (± SE) is shown (*n* = 60 leaves). Intrapopulations differences during the study period are significantly different (*Tukey* test, $p \leq 0.05$). Within the same month, differences between C_{p1} and C_{p2} and between I_{p1} and I_{p2} are significantly different (*Tukey* test, $p \leq 0.05$) except when indicated (n.s. – not significant).

Table 2. Predawn leaf water potential (Ψ_{pd}) of populations of *C. pygmaea* (C_{p1} and C_{p2} growing at 2,250 m.a.s.l. and at 2,310 m a.s.l., respectively) and *I. apennina* (I_{p1} and I_{p2} growing at 2,310 m a.s.l. and 2,350 m a.s.l., respectively) during the study period. Mean values (± SE) are shown (*n* = 20). Intrapopulations differences during the study period are significantly different (*Tukey* test, $p \leq 0.05$). Within each column different capital letters indicate significant differences between C_{p1} and C_{p2} while different lowercase letters indicate significant differences between I_{p1} and I_{p2} (*Tukey* test, $p \leq 0.05$).

Population	Ψ_{pd} [MPa] July	 August	 September
C_{p1}	-0.94 ± 0.03^A	-0.51 ± 0.05^A	-1.08 ± 0.04^A
C_{p2}	-1.32 ± 0.02^B	-0.82 ± 0.01^B	-1.40 ± 0.01^B
I_{p1}	-0.65 ± 0.02^a	-0.57 ± 0.01^a	-0.77 ± 0.01^a
I_{p2}	-0.74 ± 0.01^b	-0.62 ± 0.01^b	-0.80 ± 0.02^a

Table 3. Predawn relative water content (RWC$_{pd}$) of the populations of *C. pygmaea* (C_{p1} and C_{p2} growing at 2,250 m.a.s.l. and at 2,310 m a.s.l., respectively) and *I. apennina* (I_{p1} and I_{p2} growing at 2,310 n a.s.l. and 2,350 m a.s.l., respectively) during the study period. Mean values (± SE) are shown (*n* = 20). Within the same row *different letters* indicate significant intrapopulations differences during the study period. Within each column differences between C_{p1} and C_{p2} and between I_{p1} and I_{p2} are not significant (*Tukey* test, $p \geq 0.05$) except when indicated (*).

Population	RWC$_{pd}$ [%] July	 August	 September
C_{p1}	90 ± 1^a	93 ± 1^a	87 ± 2^a
C_{p2}	91 ± 1^a	92 ± 1^a	89 ± 1^a
I_{p1}	$83 \pm 2^{a*}$	89 ± 1^b	86 ± 1^c
I_{p2}	87 ± 1^a	92 ± 2^b	87 ± 1^a

respectively, in August (T_a = 15.4–16.5°C), and by 43 and 33%, respectively, in September (T_a = 5.4–6.5°C). I_{p1} and I_{p2} had the highest g_s in July [0.22 ± 0.03 mol(H$_2$O) m^{-2} s^{-1}, mean value] decreasing by 38 and 15% in I_{p1} and I_{p2}, respectively, in August. (Fig. 2*B*). I_{p2} had a significantly higher g_s [0.15 ± 0.01 mol(H$_2$O) m^{-2} s^{-1}] than I_{p1} [0.10 ± 0.01 mol(H$_2$O) m^{-2} s^{-1}] in September. On an average, I_{p2} had 29% higher WUE than I_{p1} (Fig. 2*C*).

Leaf respiration: C_{p1} and C_{p2} had the highest *R* in September [2.53 ± 0.10 μmol(CO$_2$) m^{-2} s^{-1}, mean value], decreasing by 33% (mean value of C_{p1} and C_{p2}) in July, and by 38% (mean value of C_{p1} and C_{p2}) in August (Fig. 2*D*). *R* of I_{p1} and I_{p2} had the same trend as C_{p1} and C_{p2}, with the highest rates in September, and the lowest ones in August. In particular, *R* of I_{p2} was 74% 69% and 22%, lower than in I_{p1} in July, August and September, respectively (Fig. 2*D*).

Gross photosynthesis: C_{p1} and C_{p2} reached the highest P_g [18.15 ± 0.36 μmol(CO$_2$) m^{-2} s^{-1}, mean value] in July, decreasing by 22 and 8%, respectively, in August, and 46

Table 4. Mean phenotypic plasticity index (PI) of *C. pygmaea* and *I. apennina* at population level (C_{p1} and C_{p2}: *C. pygmaea* growing at 2,250 m. a.s.l. and at 2,310 m a.s.l., respectively and, I_{p1} and I_{p2}: *I. apennina* growing at 2,310 m a.s.l. and 2,350 m a.s.l., respectively) and at species level, for the considered physiological (P_N, g_s, R, WUE, Chl, P_g, Ψ_{pd}, and RWC_{pd}) and morphological (LA, DM, LMA, and LTD) traits.

	PI			
Population level	C_{p1}	C_{p2}	I_{p1}	I_{p2}
Physiological traits	0.58	0.41	0.42	0.34
Morphological traits	0.32	0.38	0.31	0.29
Species level	*C. pygmaea*	*I. apennina*		
Physiological traits	0.50	0.38		
Morphological traits	0.35	0.30		

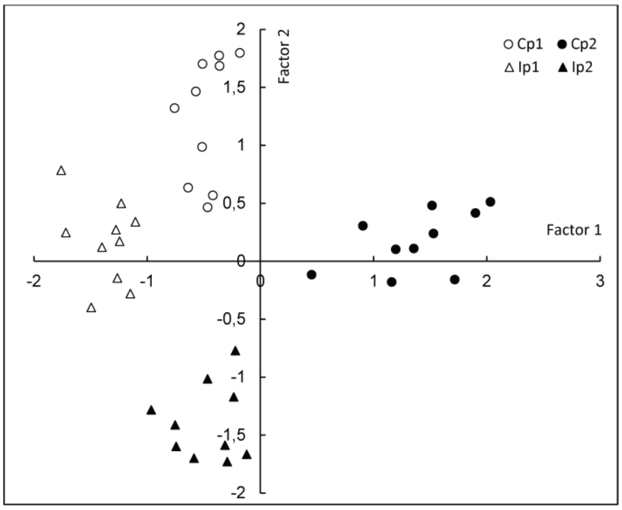

Fig. 3. Principal component analysis (PCA) carried out using the data set for *C. pygmaea* populations (C_{p1} and C_{p2} growing at 2,250 m a.s.l and at 2,310 m a.s.l, respectively) and *I. apennina* populations (I_{p1} and I_{p2} growing at 2,310 m a.s.l and at 2,350 m a.s.l, respectively). Factor 1 accounting for 45% of the total variance was significantly and positively related to P_N, Ψ_{pd}, P_g, and negatively related to LMA and LTD. Factor 2 accounting for 22% of the total variance was significantly related to IP (plasticity index).

and 37%, respectively, in September (Fig. 2*E*). I_{p1} and I_{p2} showed the same trend as P_g with the highest rates [16.55 ± 0.41 μmol(CO$_2$) m^{-2} s^{-1}, mean value] in July, decreasing by 34 and 27%, respectively, in August, and 35 and 21%, respectively, in September (Fig. 2*E*).

Chl content: C_{p1} and C_{p2} had the highest Chl (58.0 ± 1.0 SPAD units, mean value) in July, decreasing by 2% and 11% in August, respectively, and by 16 and 19%, in September, respectively (Fig. 2*F*). Chl of I_{p1} and I_{p2} was the highest in July (66.2 ± 0.4 SPAD units, mean value) decreasing by 7% (mean value) in August and in September (Fig. 2*F*).

Water relations: Ψ_{pd} of C_{p1} and C_{p2} had the same trend with the most significantly negative values in September (–1.08 ± 0.04 and –1.40 ± 0.01 MPa, respectively) (Table 2). The less negative values were monitored in August (–0.51 ± 0.05 and –0.82 ± 0.01 MPa in C_{p1} and C_{p2}, respectively).

Both I_{p1} and I_{p2} in September had the most negative Ψ_{pd} (–0.77 ± 0.01 and –0.80 ± 0.02 MPa, respectively) and the less negative values in August (–0.57 ± 0.01 and –0.62 ± 0.01 MPa, respectively) (Table 2).

RWC_{pd} did not significantly vary during the study period between C_{p1} and C_{p2} (90 ± 1 %, mean value) (Table 3). On the contrary, significantly differences in RWC_{pd} were found between I_{p1} and I_{p2} in July (83 ± 2 and 87 ± 1 %, respectively) (Table 3).

Plasticity index: C_{p1} had a significantly higher PI (0.58) of the physiological traits than C_{p2} (0.41) while the PI of the morphological traits did not significantly vary between C_{p1} and C_{p2} (Table 4). I_{p1} had a significantly higher PI for physiological and morphological traits (0.42 and 0.31, respectively) than I_{p2} (0.34 and 0.29, respectively) (Table 4). At species level, *C. pygmaea* had a significantly higher PI for both physiological and morphological traits as compared to *I. apennina* (Table 4).

Principal component analysis: PCA extracted two factors which accounted for 67% of the total variance: 45% was due to factor 1 and 22% to factor 2 (Fig. 3). The analysis showed a colinearity among the considered plant traits; in particular, factor 1 was significantly correlated to LMA (r = –0.94), P_N (r = 0.88), Ψ_{pd} (r = 0.88), P_g (r = 0.86), and LTD (r = –0.85). Factor 2 was significantly correlated to PI (r = 0.86). The analysis showed that *C. pygmaea* and *I. apennina* populations had the same trend for the considered traits (*i.e.* P_N , Ψ_{pd} and P_g increased with the altitude, while LMA and LTD decreased). Moreover, the populations of both the two species growing at the lowest altitude (C_{p1} and I_{p1}) had a higher morphological and physiological PI than the populations growing at the highest altitude (C_{p2} and I_{p2}).

Discussion

Plant trait variations in high mountain areas can change over a relatively small scale and sometimes these variations are largely genetically based, but in other cases they are mostly environmentally based (Byars *et al.* 2007). Our results on the whole underline significant differences in morphological and physiological traits of *C. pygmaea* and *I. apennina*, growing at different altitudes on the Gran Sasso Massif, at both population and species level.

At population level, with regard to morphological traits, LMA is significantly lower for both *C. pygmaea* and *I. apennina* populations growing at the highest altitude (2,310 m and 2,350 m, respectively) than for the populations growing at the lowest altitude (2,250 m and 2,310 m, respectively). LTD has the same trend as LMA, decreasing on an average by 22% from the highest to the lowest altitude.

LMA is a species-specific trait (Reich *et al.* 1991), which can be considered an index of the construction cost of the protective structures per unit of leaf area, and it is associated to carbon assimilation (Takahashi and Miyajima 2008). On an average, thinner leaves (*i.e.* low LMA leaves) have a higher carbon assimilation capacity. Taguchi and Wada (2001) underline that leaf trait variations seem to be an adaptive response to severe growth conditions allowing the maintenance of a positive carbon balance in high mountain areas. Thus, the lowest LMA and LTD of *C. pygmaea* and *I. apennina* at the highest altitude may justify their higher photosynthetic rates than at the lowest altitude. It is known, in fact, that high LMA and LTD values provide a protective function through leaf transpiration limitation (Wright *et al.* 2004). Nevertheless, large photosynthetic protective structures in the mesophyll tissues reduce net photosynthetic rates as a result of the dilution of photosynthetic tissues with nonphotosynthetic ones (Wright *et al.* 2004). Thus, the higher P_N of both the species at the highest altitude may be the result of a low internal resistance to CO_2 transfer. Moreover, the higher P_N at the highest altitude may be related to the lower Ψ_{pd} allowing water absorption from the soil, according to the results of Aasamaa *et al.* (2010), and confirmed by the highest WUE.

With regard to physiological trait variations in response to air temperature, both *C. pygmaea* and *I. apennina* have the highest P_N in the most favourable period (July), when vegetative activity is finished, and leaves are fully expanded, in a range of air temperature from 18.2 to 19.9°C. P_N decreases on an average, by 17% in *C. pygmaea* (mean value of C_{p1} and C_{p2}) and 30% in *I. apennina* (mean value of I_{p1} and I_{p2})~~,~~ respectively, in August during the flowering period, and 50 and 38%, respectively, in September, at the beginning of the senescence phase.

Photosynthetic performance is one of the most critical aspects of physiological functions for plants to respond to microenvironmental changes (Shen *et al.* 2008). Yamori *et al.* (2005) indicate that the shift of temperature dependency in high altitude mountain plants is due to change in the optimum temperature of ribulose-1,5-bisphosphate (RuBP) carboxylation. Thus, a higher activation state of Rubisco might be responsible for the highest photosynthetic rates in July of *C. pygmaea* and *I. apennina*; Chl content has the same P_N trend, with the highest content in July in both *C. pygmaea* and *I. apennina* decreasing in August and in September.

Leaf respiration of *C. pygmaea* and *I. apennina* has a different P_N trend in September, and the highest *R* might be justified by the largest energy requirement by plants during the stress period, *i.e.* when low air temperature acts negatively on the photosynthetic activity (Flexas *et al.* 2006).

P_g which integrates photosynthesis and respiration can be used to assess the combined result~~s~~ of temperature acclimation in the two physiological processes. P_g has the highest rates, in both the species, in July, decreasing 15% in *C. pygmaea* (mean value of C_{p1} and C_{p2}) and 31% in *I. apennina* (mean value of I_{p1} and I_{p2}) in August, and 42% in *C. pygmaea* (mean value of C_{p1} and C_{p2}) and 28% in *I. apennina* (mean value of I_{p1} and I_{p2}) in September.

At the species level, our results underline that *C. pygmaea* has a significantly higher PI of both physiological and morphological leaf traits than *I. apennina*. Snow-bed plant species are phenotypically plastic, especially in size-related traits (McGraw 1987), and altitudinal variations represent a particularly useful tool to test plant traits for their long-term adaptive nature (Shi *et al.* 2010). If the plastic phenotype buffers individuals against short-term environmental fluctuations, it might be expected that phenotypic plasticity could buffer individuals against the long-term effects of climate change (Jump and Peñuelas 2005). Moreover, the results of PCA show the existence of an interpopulation difference. In particular, the main variation trend (*i.e.* factor 1) is due to the combination of physiological (P_N, P_G, and Ψ_{pd}) and morphological traits (LMA and LTD) mainly related to carbon acquisition that underlines how the populations of *C. pygmaea* and *I. apennina* growing at the highest altitude have a large carbon acquisition capacity. The residual part of the total variance (explained by factor 2) is related to the capacity of the populations to respond to environmental changes through phenotypic plasticity. In fact, C_{p1} and I_{p1} have a significantly higher PI both at physiological and at morphological levels than C_{p2} and I_{p2} underlining the potential of the two wild populations growing at the lowest altitude, to move up in response to the hypothesised air temperature increasing. This result indicates that plasticity may play an important role for the species in the acclimatization to microenvironmental conditions. Understanding adaptation capability is important particularly for threatened species such as *C. pygmaea* and *I. apennina* growing along a narrow altitudinal range. Such species may be more especially prone to extinction risk if they are unable to adapt to rapid environmental changes. Global warming could drive *C. pygmaea* and *I. apennina* to higher altitudes in the Gran Sasso Massif, with *C. pygmaea* being favored as it as the higher PI.

The functional traits explaining most of the total variance could be used to monitor *C. pygmaea* and *I. apennina* wild populations over the long term. In particular, since the photosynthetic capacity is the major determinant of growth and seedling size (Bonito *et al.*

2011), it can help in the understanding of the potential altitudinal distribution of these species in response to global change. Further researches, including long-term monitoring, phenology and seed germination capability studies, are needed to establish the competitive capacity of *C. pygmaea* and *I. apennina* with other species from the same area which might migrate from the lowest to the highest altitudes of the Gran Sasso Massif.

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