

## The cost of stress resistance: construction and maintenance costs of leaves and roots in two populations of *Quercus ilex*

RAQUEL G. LAUREANO,<sup>1</sup> YALÍN O. LAZO,<sup>1</sup> JUAN C. LINARES,<sup>1</sup> ALFREDO LUQUE,<sup>1</sup> FELICIANO MARTÍNEZ,<sup>1</sup> JOSÉ I. SECO<sup>1</sup> and JOSÉ MERINO<sup>1,2</sup>

<sup>1</sup> Department of Physical, Chemical and Natural Systems, University Pablo Olavide, Carretera de Utrera, Km 1, 41013 Seville, Spain

<sup>2</sup> Corresponding author (jamerort@upo.es)

Received March 4, 2008; accepted June 17, 2008; published online September 2, 2008

**Summary** We tested whether growth and maintenance costs of plant organs vary with environmental stress. *Quercus ilex* L. seedlings from acorns collected from natural populations in the northern Iberian Peninsula and in a lower elevation and putatively less stressful habitat in the southern Iberian Peninsula were grown in pots under the same conditions. Growth and maintenance respiration were measured by CO<sub>2</sub> exchange. Young leaves from 5-month-old seedlings of both populations had similar mean specific leaf areas, nitrogen and carbon concentrations and specific growth rates, and almost identical growth costs (1.26 g glucose g<sup>-1</sup>). Leaf maintenance cost was higher in northern than in the southern population (27.3 versus 22.4 mg glucose g<sup>-1</sup> day<sup>-1</sup>,  $P < 0.01$ ). In both populations, leaf maintenance cost decreased by 90% as leaves aged, but even in mature leaves, the maintenance cost was higher in the northern population than in the southern population (3.38 versus 2.53 mg glucose g<sup>-1</sup> day<sup>-1</sup>,  $P < 0.01$ ). The growth costs of fine roots < 1 mm in diameter were similar in the two populations (1.20 g glucose g<sup>-1</sup>), whereas fine root maintenance cost was higher in the northern population than in the southern population (9.86 versus 7.45 mg glucose g<sup>-1</sup> day<sup>-1</sup>;  $P < 0.05$ ). The results suggest that the cost of organ maintenance is related to the severity of environmental stress in the native habitat. Because the observed differences in both leaves and roots were constitutive, the two populations may be considered ecotypes.

**Keywords:** evergreen leaves, growth respiration, maintenance respiration, Mediterranean species, respiration–nitrogen relationships, root respiration, sclerophylly.

### Introduction

The costs of growth and maintenance of plant organs are a major component of both individual carbon balance and ecosystem production. Growth cost includes the energy content of the constituents of new tissue plus the energetic costs of synthetic processes, whereas maintenance cost is the energy invested in processes that result in no net increase in biomass; for example, maintenance of ion gradients, synthesis and replacement of degraded organic molecules, or processes of physiological adjustment to changing environments (acclima-

tion) (Penning de Vries et al. 1974). The relative importance of these processes, and the chemical composition of leaf tissue, may vary among populations of the same species depending on environmental conditions; for example, as a function of the degree of stress (Martínez et al. 2002a). This may result in differences in the carbon balance of individuals, thereby determining the presence or absence of populations in a given area (i.e., species distribution).

Highly stressed populations, such as those in mountain areas, or those exposed to high irradiances or to atmospheric contaminants, display significantly higher concentrations of defense and repair complexes than populations subjected to more moderate stress (Dixon et al. 2005). The greater abundance of metabolic defense and repair complexes in the tissues of stressed individuals increase both tissue construction costs, and because of the increased complexity of the metabolic machinery and increased rates of tissue repair and detoxification, maintenance costs. The higher rates of leaf respiration reported for species growing in stressful environments compared with species in less stressful environments (Wright et al. 2006) support this assumption, as does the higher resistance to SO<sub>2</sub> fumigation of seedlings of *Quercus ilex* L. from putatively more stressful northern habitats than that of seedlings from southern habitats (García et al. 1998).

Certain differences among populations in defense and repair mechanisms appear to be constitutive (Lorenzini et al. 2002). This may account for the finding that, when grown in a common low-stress environment (gardens, growth chambers), populations native to more stressful environments generally display higher respiration rates (growth or maintenance, or both) than populations from less stressful environments; for example, arctic versus temperate (Lechowicz et al. 1980, Earnshaw 1981) or alpine versus lowland (Mooney 1963, Mariko and Koizumi 1993).

Several studies have focused on respiration patterns in populations native to both latitudinal and altitudinal gradients growing under similar experimental conditions; but with few conclusive results (for a summary see Wright et al. 2006). The lack of a clear pattern in respiratory trends might be linked to methodological limitations. For example, in young growing organs, high rates of growth respiration may mask variation in

rates of maintenance respiration, whereas in mature organs, variation in maintenance respiration rates may be masked by high respiration rates associated with processes other than maintenance (Cannell and Thornley 2000), thus blurring the stress resistance–maintenance respiration relationships. A further limitation of most previous studies is that they omitted observation of belowground plant parts. Finally, many earlier studies considered only extreme climatic environments (e.g., arctic versus temperate, alpine versus lowland), so information is lacking for species from other ecosystems.

*Quercus ilex* is a common evergreen tree species in Mediterranean landscapes; it grows as far north as 43°30' N, and southward along the West African coastline as far as Agadir (Morocco) (30°30' N). The stressful nature of the Mediterranean climate, in which cold wet winters alternate with hot dry and sunny summers, has led to the development of biochemical defense systems in the leaves of this species (Corcuera et al. 2005). The wide distribution of *Q. ilex* includes an immense variety of climates and soils that result in a diversity of habitats presenting differing degrees of stress. The selective pressures generated by this diversity of habitats may in turn lead to the existence of a mosaic of populations, differing from each other in defense and repair capability, and therefore in tissue growth and maintenance costs.

We compared the growth and maintenance costs of leaf and root tissues of *Q. ilex* individuals from the north of the Iberian Peninsula with those of individuals from the south of the peninsula, grown in uniform conditions. Growth and maintenance components were separated as described by Hesketh et al. (1971). Though this approach is not exempt from criticism (cf. Hansen et al. 2002), it has been judged adequate for comparative purposes (Cannell and Thornley 2000). We postulated that growth and maintenance costs are higher in northern individuals than in southern individuals, because environmental stress is greater in the northern habitats and leads to higher concentrations of constitutive tissue defense and repair complexes.

## Materials and methods

Two *Q. ilex* populations were selected for study: one located in the north of the Iberian Peninsula (42°25' N, 06°03' W, 850 m a.s.l.), and the other in the south (37°50' N, 06°00' W, 400 m a.s.l.); the distance between sites is about 650 km. The northern site differed from the southern site in mean annual temperature (10.8 versus 17.0 °C), mean minimum temperature of the coldest month (−1.4 versus 3.7 °C), mean number of months with frosts (2.5 versus 0.0 months) and mean annual rainfall (542 versus 857 mm).

### Leaves

Acorns were collected from 50 trees of each population (about 12 acorns per tree) and taken to the laboratory, where they were pooled by population and placed in trays for germination. One month after germination, seedlings in poor condition were discarded and 50 seedlings of each population were selected and each placed in a 2-l pot containing a 1:1 (v/v) ver-

miculite:sand substrate. Seedlings of both populations were placed in each of two growth chambers providing the following conditions: 14-h photoperiod; photosynthetic photon flux (PPF) at plant height of 325  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; day/night temperatures of 24/18 °C; and a relative humidity of 30–35%. Plants were watered on alternate days with diluted (1:3) Hoagland solution (Epstein 1972) to avoid nutrient deficiency and water stress. To minimize chamber effects, plants were rotated both inside the chamber (about every 4 days) and between chambers (about every 10 days). Unless otherwise indicated, respiration and growth rates were measured on attached leaves of 5-month-old seedlings. Linear and exponential regression models of leaf dry mass versus age were established for each population.

The growth rate of each leaf was estimated over a 3-day period. Leaf surface area was measured on Day 1 and Day 3 using paper images. The leaf was removed on Day 3, washed, dried at 80 °C, weighed, and the specific leaf area (SLA) determined as the ratio of leaf area to leaf dry mass. The SLA did not change over the 3-day period and was used to estimate the increase in leaf mass over the period, which was used to calculate the specific growth rate (SGR) as the difference in  $\ln(\text{mass})$  divided by days of growth.

On Day 2, total respiration ( $R_t$ ) was measured as the flow of  $\text{CO}_2$  between the leaf and the atmosphere in darkness at 20 °C, with a portable gas exchange system similar to that described by Field et al. (1982). Measurements were continued until a stable respiration rate was achieved (less than 60 min). The specific respiration rate (SRR) for each leaf was calculated by dividing the measured respiration rate by mean leaf mass over the period. Measurements were made for a total of 71 and 73 leaves from 44 and 41 seedlings (about two leaves per seedling) of the northern and southern populations, respectively. A linear regression of SRR against SGR was performed for each population. The slope ( $\text{mg CO}_2 \text{g}^{-1}$ ) represents respiration associated mainly with tissue synthesis (growth respiration,  $R_g$ ), and the y-intercept ( $\text{mg CO}_2 \text{g}^{-1} \text{day}^{-1}$ ) represents the respiration rate at zero growth, i.e., respiration associated mainly with tissue maintenance (maintenance respiration,  $R_m$ ) (Hesketh et al. 1971, Thornley and Cannell 2000).

Maintenance respiration was also estimated by quantifying respiration in mature fully expanded leaves, assuming that, in the absence of leaf growth, total respiration was largely associated with maintenance processes. Estimation was based on leaves around 10 months old, from 32 seedlings selected at random from those used in the previous experiments. Total respiration of each attached leaf was estimated as previously described. We measured 32 leaves (one per plant) for each population, and the mean of all measurements was taken as the leaf maintenance respiration for the population.

### Roots

Acorns of uniform size were selected and placed on moist sterile sand to induce radicle emergence. After about 20 days, when the root was 6 cm long, it was transferred to a hydroponic medium in a growth chamber. The day of transfer was

taken as seedling age zero. The hydroponic system comprised 100-l tanks containing nutrient solution that was aerated with two 5-W air compressors. The growth medium was renewed frequently to avoid nutrient depletion.

Growth rates of root systems in each population were estimated at eight harvests, corresponding to eight age classes. Every 3 days, the complete root systems of 5–10 seedlings were collected. The root was separated from the shoot, washed, dried at 80 °C for 48 h and weighed, to obtain the dry mass of the whole-seedling root system. For each age-class considered, the mean root mass of all sampled seedlings was estimated. In total, 91 and 88 root systems were measured for the northern and southern populations, respectively. Linear and exponential regression models of dry root mass versus age were established for each population. These growth equations were used to calculate the SGR of the seedlings for which SRR was estimated.

Root respiration of intact seedlings was measured as oxygen uptake with the open continuous-flow system described by Martínez et al. (2002b). The system consisted of an open circuit connected to a container of nutrient solution. The circuit included a chamber to house the root system of a 25-cm-tall seedling and an oxygen electrode (Hansatech, U.K.) to measure the concentration of dissolved oxygen in the chamber solution. During the experiments, the root chamber was kept in darkness at 20 °C, whereas the aboveground portion of the seedling was illuminated at a PPF of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at a constant temperature of about 23 °C; these conditions were almost identical to the growing conditions. Respiration was measured at ages ranging from 2 to 24 days, in 28 and 29 seedlings of the northern and southern populations, respectively. After each respiration measurement, roots were separated, washed, dried at 80 °C and weighed.

### Costs

Construction cost was estimated as the sum of the energy used in growth respiration plus the energy stored in the form of tissue mass increase during organ growth. Values were expressed in glucose equivalents ( $\text{g glucose g}^{-1}$  dry mass). The intercept of regression lines with the y-axis (maintenance respiration) was expressed in  $\text{mg glucose g}^{-1}$  dry mass  $\text{day}^{-1}$ .

### Carbon and nitrogen concentrations

The study leaves (young, mature and intermediate; the latter were used to analyze the effect of temperature—see below) and root systems were ground individually. The nitrogen and total carbon concentrations in the ground tissue of each organ were measured with an elemental analyzer (LECO Corporation, St. Joseph, MI). Nitrogen concentration was expressed as  $\text{mg nitrogen g}^{-1}$ , and carbon concentration as a percentage of total mass. A total of 106 and 109 leaves plus 28 and 29 root systems were assayed for the northern and southern populations, respectively. For each population, the relationship of ln-transformed respiration versus nitrogen concentration was analyzed by linear regression.

### Respiration response to temperature

To assess the effect of temperature on respiration, a leaf of intermediate age was selected from each of four seedlings from the southern population and from each of three seedlings from the northern population. The total respiration of each attached leaf was measured at six temperatures from 2 to 35 °C. The experiment was repeated for the attached whole root systems of eight individuals (four from each population) over the temperature range 10 to 30 °C. For each population, the linear regression of organ respiration versus temperature was established.

### Statistical analysis

To estimate root growth equations for each population, root mass was regressed against age by both linear and exponential models. To estimate growth and maintenance respiration ( $R_g$  and  $R_m$ , respectively), SRRs were regressed (linear model) against SGRs. To detect differences in  $R_g$  and  $R_m$  between populations, regression lines for SRR versus SGR were subjected to a parallelism test and to Tukey's test (Zar 1999), and comparison of the other parameters studied was by one-way analysis of variance (ANOVA) with population as a factor.

## Results

### Growth and respiration of young and mature leaves

Analysis of variance for mass–age regressions showed that the growth of young leaves fitted the linear model ( $P < 0.001$ ) better than the exponential model (data not shown). Young leaves of both populations had similar mean leaf size (LS), SGR, SLA and carbon and nitrogen concentrations (Table 1). Despite this uniformity, however, the mean specific respiration rate was higher in the northern population than in the southern population ( $P < 0.05$ ).

Specific respiration rates correlated positively with specific growth rates of young leaves for both populations (Figure 1). Maintenance respiration was higher in the northern population than in the southern population ( $40.1 \pm 4.9$  versus  $32.8 \pm 3.6$   $\text{mg CO}_2 \text{g}^{-1} \text{day}^{-1}$ ;  $P < 0.01$ ), whereas there was no significant difference in  $R_g$  between the populations (Table 2).

Mature leaves of both populations had similar nitrogen concentrations and LS, but SLA was greater ( $P < 0.05$ ) in the southern population than in the northern population (Table 3). Compared with the southern population,  $R_t$  was higher ( $P < 0.01$ ) in the northern population, which was equivalent to a leaf  $R_m$  of  $4.96 \pm 0.26$   $\text{mg CO}_2 \text{g}^{-1} \text{day}^{-1}$ ; whereas the corresponding value in the southern population was  $3.72 \pm 0.23$   $\text{mg CO}_2 \text{g}^{-1} \text{day}^{-1}$ .

Growth respiration per unit mass in young leaves was virtually identical in the study populations (mean:  $151 \text{ mg CO}_2 \text{g}^{-1}$ , Table 2), as was carbon concentration (mean: 45.3%, Table 1); therefore, the growth cost of the study populations was similar at  $1.26 \text{ g glucose g}^{-1}$  (Table 2). Compared with the southern population,  $R_m$  was higher in the northern population and therefore so was the maintenance cost ( $27.3 \pm 3.3$  versus  $22.4 \pm 2.4$   $\text{mg glucose g}^{-1} \text{day}^{-1}$ ,  $P < 0.01$ ). Similar results were obtained for mature leaves (Table 3): maintenance costs were

Table 1. Means ( $\pm$  standard error) of variables measured in young leaves of northern and southern populations of *Quercus ilex*. Asterisks (\*) denote significant differences between populations ( $P < 0.05$ ). Abbreviations:  $n$  = number of sampled leaves; LS = leaf size; SLA = specific leaf area; [C] = carbon concentration; [N] = nitrogen concentration; SGR = specific growth rate; and SRR = specific respiration rate.

Population	$n$	LS (cm <sup>2</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )	[C] (%)	Carbon cost (g Glu g <sup>-1</sup> )	[N] (mg g <sup>-1</sup> )	SGR (mg g <sup>-1</sup> day <sup>-1</sup> )	SRR (mg CO <sub>2</sub> g <sup>-1</sup> day <sup>-1</sup> )
Northern	71	2.6 $\pm$ 0.2	124 $\pm$ 2	45.1 $\pm$ 0.8	1.18 $\pm$ 0.03	20 $\pm$ 0.5	115 $\pm$ 7	57.1 $\pm$ 2.3 *
Southern	73	2.8 $\pm$ 0.2	123 $\pm$ 3	45.5 $\pm$ 1.0	1.14 $\pm$ 0.02	20 $\pm$ 0.5	105 $\pm$ 7	49.0 $\pm$ 2.1 *

higher in the northern population than in the southern population (3.38  $\pm$  0.17 versus 2.53  $\pm$  0.15 mg glucose g<sup>-1</sup> day<sup>-1</sup>,  $P < 0.01$ ).

#### Growth and respiration in root systems

Mass–age regressions in root systems fit ( $P < 0.0001$ ) the linear model in both populations. On the basis of these linear equations, and for each population, the SGRs (mg g<sup>-1</sup> day<sup>-1</sup>) of root systems were calculated for each age interval for which SRR was estimated. The mean SGRs of the root systems considered were similar in both populations (Table 4), whereas SRR was higher ( $P < 0.01$ ) in the northern population than in the southern population.

The observed SRRs in seedlings of different ages were re-

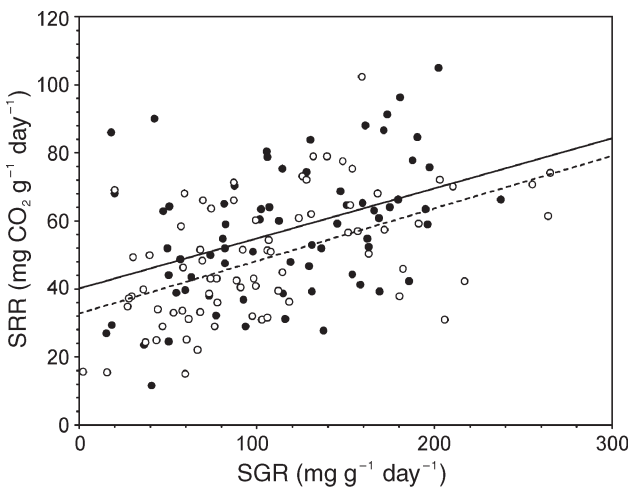


Figure 1. Specific respiration rate (SRR) versus specific growth rate (SGR) in young leaves of northern and southern populations of *Quercus ilex*. Regression lines are given in Table 2. Northern population: solid line and filled symbols ( $r = 0.41$ ,  $P < 0.01$ ). Southern population: broken line and open symbols ( $r = 0.52$ ,  $P < 0.01$ ).

Table 2. Regression equations for specific respiration rate (SRR, mg CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>) versus specific growth rate (SGR, mg g<sup>-1</sup> day<sup>-1</sup>) in young leaves of northern and southern populations of *Quercus ilex*. Asterisks (\*\*) denote significant differences between populations ( $P < 0.01$ ). Abbreviations:  $n$  = number of sampled leaves;  $R_g$  = growth respiration; and  $R_m$  = maintenance respiration.

Population	$n$	Equation	$R_g$ (mg CO <sub>2</sub> g <sup>-1</sup> )	$R_m$ (mg CO <sub>2</sub> g <sup>-1</sup> day <sup>-1</sup> )	Growth cost (g Glu g <sup>-1</sup> )	Maintenance cost (mg Glu g <sup>-1</sup> day <sup>-1</sup> )
Northern	71	SRR = 0.15(SGR) + 40.1	147 $\pm$ 39	40.1 $\pm$ 4.9 **	1.28 $\pm$ 0.02	27.3 $\pm$ 3.3 **
Southern	73	SRR = 0.15(SGR) + 32.8	154 $\pm$ 30	32.8 $\pm$ 3.6 **	1.24 $\pm$ 0.03	22.4 $\pm$ 2.4 **

gressed against their SGRs (Figure 2). In both populations, the significance of the regression line was  $P < 0.01$ . The regression equations yielded  $R_g$  and  $R_m$  for the two populations (Table 5). Maintenance respiration was higher in the northern population than in the southern population (329  $\pm$  18 versus 248  $\pm$  7  $\mu$ mol O<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>,  $P < 0.01$ ), whereas there was no significant difference between populations in growth respiration (mean = 4.68 mmol O<sub>2</sub> g<sup>-1</sup>).

Root nitrogen concentrations in the two populations were similar (mean = 31 mg g<sup>-1</sup>, Table 4), as were carbon concentrations (mean = 42.1%); consequently, carbon skeleton costs were similar in the two populations (mean = 1.06 g glucose g<sup>-1</sup>, Table 4). Mean  $R_g$  (Table 5) was 0.14 g glucose g<sup>-1</sup>. Thus, the growth costs for the two populations were almost identical (mean = 1.20 g glucose g<sup>-1</sup>, Table 5). Maintenance respiration was higher ( $P < 0.05$ ) in the northern population than in the southern population (Table 5) and, therefore, so was the maintenance cost (9.86  $\pm$  0.53 versus 7.45  $\pm$  0.20 mg glucose g<sup>-1</sup> day<sup>-1</sup>).

#### Effects of tissue nitrogen concentration and temperature on respiration

A positive relationship was noted between SRR and nitrogen concentration when considering all leaves in the study (young, intermediate and mature) (Figure 3). The populations behaved in a similar fashion (no significant difference between regression lines, data not shown), displaying a clear decline in respiration rates associated with decreasing nitrogen concentrations ( $P < 0.001$  in both populations). There was no significant relationship between nitrogen concentration and SRR in young roots.

Leaf respiration values for the two populations were similar for each temperature considered (Figure 4). In roots, the pattern of response was the same in both populations, with slightly higher respiration rates in the northern population than in the southern population. No significant difference between regression lines, in either slope or y-intercept, was detected.



Table 3. Means ( $\pm$  standard error) of variables measured in mature leaves of northern and southern populations of *Quercus ilex*. Significant differences between populations are indicated: \*,  $P < 0.05$ ; and \*\*,  $P < 0.01$ . Abbreviations:  $n$  = number of sampled leaves; LS = leaf size; SLA = specific leaf area; [N] = nitrogen concentration; and  $R_t$  = total respiration.

Population	$n$	LS (cm <sup>2</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )	[N] (mg g <sup>-1</sup> )	$R_t$ (mg CO <sub>2</sub> g <sup>-1</sup> day <sup>-1</sup> )	Maintenance cost (mg Glu g <sup>-1</sup> day <sup>-1</sup> )
Northern	32	7.2 $\pm$ 0.2	50.2 $\pm$ 1.2 *	12 $\pm$ 0.3	4.96 $\pm$ 0.26 **	3.38 $\pm$ 0.17 **
Southern	32	6.8 $\pm$ 0.3	60.3 $\pm$ 1.3 *	11 $\pm$ 0.4	3.72 $\pm$ 0.23 **	2.53 $\pm$ 0.15 **

Table 4. Means ( $\pm$  standard error) of variables measured in young root systems of northern and southern populations of *Quercus ilex*. Asterisks (\*\*) indicate significant differences between populations ( $P < 0.01$ ). Abbreviations:  $n$  = number of sampled root systems; [C] = carbon concentration; [N] = nitrogen concentration; SGR = specific growth rate; and SRR = specific respiration rate.

Population	$n$	[C] (%)	Carbon cost (g Glu g <sup>-1</sup> )	[N] (mg g <sup>-1</sup> )	SGR (mg g <sup>-1</sup> day <sup>-1</sup> )	SRR ( $\mu$ mol O <sub>2</sub> g <sup>-1</sup> day <sup>-1</sup> )
Northern	28	42.4 $\pm$ 1.0	1.06 $\pm$ 0.02	30 $\pm$ 0.6	161 $\pm$ 14	1112 $\pm$ 80 **
Southern	29	41.8 $\pm$ 1.2	1.05 $\pm$ 0.03	31 $\pm$ 1.4	143 $\pm$ 15	711 $\pm$ 67 **

## Discussion

### Growth cost

The results did not support the hypothesis that organ growth cost is higher in northern populations than in southern populations. The mean organ growth cost of 1.26 g glucose g<sup>-1</sup> for young leaves of *Quercus ilex* was lower than that reported by Villar and Merino (2001) for mature leaves of *Q. ilex* growing in natural conditions. The mean root growth cost (1.20 g glucose g<sup>-1</sup>) was also lower than the values reported by Martínez et al. (2002a) for young roots of seven *Quercus* species (including *Q. ilex*) growing in natural conditions in SW Spain, but comparable with the values of the same seven species growing in hydroponic cultures (Martínez et al. 2002b).

Growth conditions partly determine the chemical composition of tissues (Martínez et al. 2002a, Villar et al. 2006). In less stressful environments, selection tends to favor tissues proportionally richer in cellulose (a low-cost component) and with a lower wax content (a costly component) (Martínez et al. 2002a). The favorable growth conditions in our study (no water or nutrient limitations) may account for the low growth costs observed for both leaves and roots. The absence of a significant difference in growth cost between our study populations suggests that there was no difference in the chemical composition of leaf tissues, and thus no difference in defense and repair complex concentrations between populations (but see below).

### Maintenance cost

The results supported the hypothesis that the northern population expends more energy on maintenance than the southern population. Despite considerable differences associated with

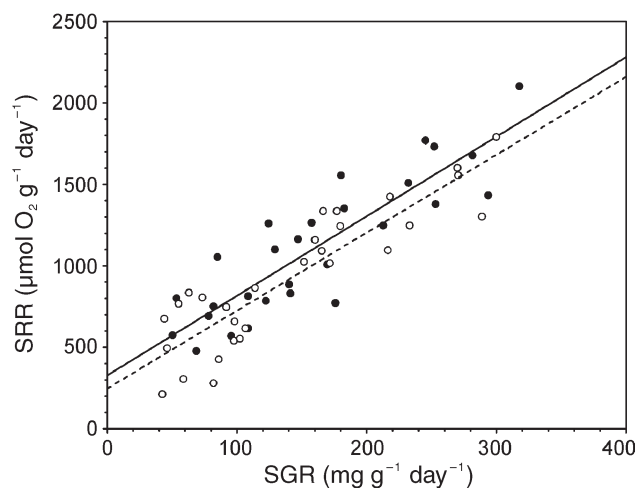


Figure 2. Specific respiration rate (SRR) versus specific growth rate (SGR) in young roots of northern and southern populations of *Quercus ilex*. Equations were derived from the regressions given in Table 5. Northern population: solid line and filled symbols ( $r = 0.87$ ,  $P < 0.01$ ). Southern population: broken line and open symbols ( $r = 0.89$ ,  $P < 0.01$ ).

Table 5. Regression equations for specific respiration rate (SRR, mg CO<sub>2</sub> g<sup>-1</sup> day<sup>-1</sup>) versus specific growth rate (SGR, mg g<sup>-1</sup> day<sup>-1</sup>), in young roots of northern and southern populations of *Quercus ilex*. Significant differences between populations are indicated: \*,  $P < 0.05$ ; and \*\*,  $P < 0.01$ . Abbreviations:  $n$  = number of sampled root systems;  $R_g$  = growth respiration; and  $R_m$  = maintenance respiration.

Population	$n$	Equation	$R_g$ (mmol O <sub>2</sub> g <sup>-1</sup> )	$R_m$ ( $\mu$ mol O <sub>2</sub> g <sup>-1</sup> day <sup>-1</sup> )	Growth cost (g Glu g <sup>-1</sup> )	Maintenance cost (mg Glu g <sup>-1</sup> day <sup>-1</sup> )
Northern	28	SRR = 4.88(SGR) + 329 *	4.88 $\pm$ 0.54	329 $\pm$ 18 **	1.21 $\pm$ 0.04	9.86 $\pm$ 0.53 *
Southern	29	SRR = 4.48(SGR) + 248 *	4.48 $\pm$ 0.68	248 $\pm$ 7 **	1.18 $\pm$ 0.05	7.45 $\pm$ 0.20 *

either organ type or age, maintenance costs of roots and leaves in the northern population were significantly higher than in the southern population (Tables 2, 3 and 5).

Population differences in organ maintenance costs were observed even though seedlings of the two populations were grown under similar conditions, and there were no significant differences between populations for variables relating to leaf structure, chemical composition (nitrogen and carbon concentrations) and growth rates in either young leaves or roots (Tables 1 and 4). In all cases, total respiration was measured at the growth temperature, thus excluding any stimulation effect. Moreover, the pattern of response of total respiration to temperature was similar in the two populations (Figure 4), indicating that the differences observed in  $R_m$  did not result from a differential response to growth temperature (Tjoelker et al. 1999, Atkin et al. 2006, Zaragoza-Castells et al. 2007). Therefore, the differences detected in maintenance cost can be ascribed to intrinsic (genetic) differences between the populations, which might thus be classed as ecotypes (Oleksyn et al. 1998). A clear genetic component of respiration rate has been demonstrated in varieties of some tree species (Callister et al. 2007).

Our results may appear contradictory because, despite the significant differences observed in maintenance cost between populations, there were no differences in growth cost. A similar lack of agreement has been reported both among ecotypes native to different altitudes when grown together under common conditions (Mariko and Koizumi 1993), and among treatments in ozone-fumigation experiments (Amthor and Cummings 1988). However, the contradiction may be in appearance only, because the supplementary biochemical machinery for defense and repair is present in plant tissues at low concentrations (e.g., Grill et al. 2001); thus its contribution to total growth cost may be negligible, although it may have a high turnover, thus contributing significantly to the maintenance cost.

#### Effect of leaf age

The marked difference in maintenance costs between young and mature leaves within the same population may be related

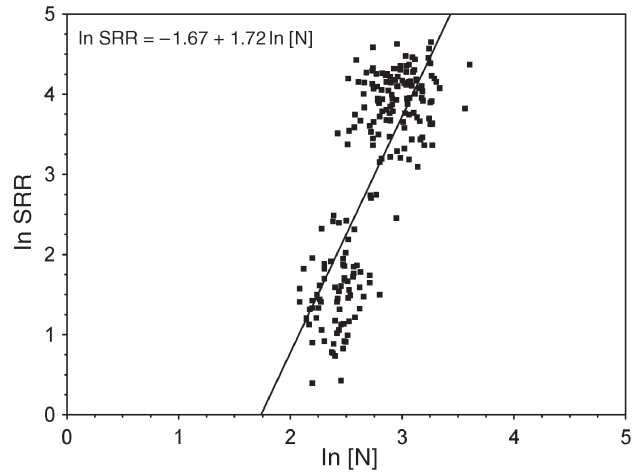


Figure 3. Specific respiration rate (SRR,  $\text{mg CO}_2 \text{ g}^{-1} \text{ day}^{-1}$ ) for each leaf plotted against its nitrogen concentration ( $[\text{N}]$ ,  $\text{mg g}^{-1}$ ) ( $r = 0.73$ ,  $P < 0.001$ ).

to protein concentration, given the significant ( $P < 0.001$ ) difference in nitrogen concentration between leaves of different ages (Tables 1 and 3). Proteins are the major component of the leaf maintenance cost (Penning de Vries 1975), not only because of their high specific maintenance cost and their abundance (about 12% of the dry mass of young *Q. ilex* leaves; Table 1), but because of the positive correlation between protein concentration and the abundance of tissue metabolic machinery (Bouma et al. 1994). All of these factors could account for the observed significant correlation between leaf nitrogen concentration and total respiration rate (Figure 3), as already noted for several species (Ryan 1995, Ryan et al. 1996, Wright et al. 2004, Reich et al. 2006), including *Quercus* ssp. (Martínez et al. 2002b, Xu and Griffin 2006).

The tenfold decrease in maintenance cost associated with leaf aging was more marked than the decrease (50%) in nitrogen concentration (Tables 1–3). The decline in maintenance respiration can be ascribed not only to a dilution of metabolic machinery through an increase in structural components (cellulose, lignin, etc.) resulting from a roughly 50% decrease in

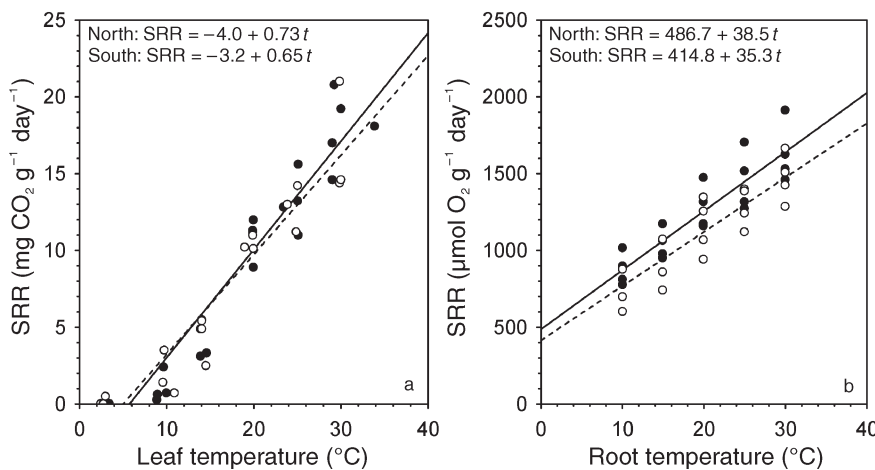


Figure 4. (a) Response of leaf specific respiration rate (SRR) to temperature in northern and southern populations of *Quercus ilex*. Northern population: solid line and filled symbols ( $r = 0.96$ ,  $P < 0.001$ ), southern population: broken line and open symbols ( $r = 0.95$ ,  $P < 0.001$ ). (b) Response of root specific respiration rate (SRR) to temperature in northern and southern populations of *Q. ilex*. Northern population ( $r = 0.89$ ,  $P < 0.001$ ), southern population ( $r = 0.88$ ,  $P < 0.001$ ).

SLA with age (Tables 1 and 3), but also to the existence of various nitrogen fractions (structural, enzymatic, reserve), each with a different turnover rate and thus a different specific maintenance cost. For example, as suggested by Figure 3, a nitrogen fraction (either a reserve or a structural fraction) accounting for about 30% of the nitrogen concentration of young leaves makes no appreciable contribution to respiration and so its contribution to tissue maintenance cost is nil.

The existence of nitrogen fractions differing in degree of activity and the change in their relative proportions over either time or space have been found responsible for the lack of statistical significance between tissue nitrogen concentrations and respiration rates (Lusk and Reich 2000, Ögren 2000, Vose and Ryan 2002). In our study, a change in the proportion of the various nitrogen forms with age might account for the strong decrease in maintenance respiration, despite the comparatively small decrease in nitrogen concentrations.

Similarly, although there was no difference in nitrogen concentration between our study populations, the higher maintenance costs in roots and young and mature leaves in the northern population compared with the southern population may be the result of a comparatively greater investment in active nitrogen fractions; for example those associated with biochemical tissue defense and repair complexes. Alternatively, the difference in maintenance respiration between populations may reflect a metabolic adaptation to the length of the growing season in their respective habitats. The shorter growing season in the northern habitat would select for a more active metabolism to allow more rapid completion of the phenological cycle (Mariko and Koizumi 1993). Mean thiol concentration was higher ( $P < 0.05$ ) in twigs of *Q. ilex* individuals from the northern population than from the southern population (Laureano and De Kock, unpublished data), which supports our conclusion.

It is important to ascertain the extent to which the observed differences in maintenance cost are significant for growth. García et al. (1998), in a study of *Q. ilex* seedlings from the populations we studied, which were cultivated in similar conditions, noted that although photosynthetic rates and SLA were similar in the two populations, and although northern seedlings allocated comparatively more ( $P < 0.05$ ) biomass to aerial fractions (shoot/root, leaf area ratio), their growth rate was lower ( $P < 0.05$ ). The greater expenses in leaf and root maintenance demonstrated in our study in the northern seedlings may offset the advantage of their greater aerial biomass, resulting in the lower growth rate observed by García et al. (1998). Thus, even though the absolute differences in maintenance cost observed between the populations are small, they appear to be significant for plant growth.

#### Acknowledgments

The authors are grateful to the Ecology Department at the University of León (Spain), particularly to Dr. Leonor Calvo, for their help with acorn sampling, and to Jesús Rodríguez for assistance with gas exchange measurements. This research was partly funded by the Spanish Ministry of Science and Technology (Project REN2003-09509-CO2-O2).

#### References

- Amthor, J.S. and J.R. Cummings. 1988. Low levels of ozone increase bean leaf maintenance respiration. *Can. J. Bot.* 66:724–726.
- Atkin, O.K., I. Scheurwater and T.L. Pons. 2006. High thermal acclimation potential of both photosynthesis and respiration in two lowland *Plantago* species in contrast to an alpine congeneric. *Global Change Biol.* 12:500–515.
- Bouma, T.J., R. de Visser, J.H.J.A. Janssen, M.J. de Kock, P.H. van Leeuwen and H. Lambers. 1994. Respiratory energy requirements and rate of protein turnover in vivo determined by the use of an inhibitor of protein synthesis and a probe to assess its effect. *Physiol. Plant.* 94:585–594.
- Callister, A.N., P.K. Ades, S.K. Arndt and M.A. Adams. 2007. Clonal variation in shoot respiration and tree growth of *Eucalyptus* hybrids. *Can. J. For. Res.* 37:1404–1413.
- Cannell, M.G.R. and J.H.M. Thornley. 2000. Modeling the components of plant respiration: some guiding principles. *Ann. Bot.* 85:45–54.
- Corcuera, L., F. Morales, A. Abadía and E. Gil-Pelegrín. 2005. Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. *Tree Physiol.* 25:599–608.
- Dixon, D.P., M. Skipsey, N.M. Grundy and R. Edwards. 2005. Stress-induced protein S-glutathionylation in *Arabidopsis*. *Plant Physiol.* 138:2233–2244.
- Earnshaw, M.J. 1981. Arrhenius plots of root respiration in some arctic plants. *Arct. Alp. Res.* 13:425–430.
- Epstein, E. 1972. Mineral nutrition of plants. Principles and perspectives. John Wiley & Sons, New York, 412 p.
- Field, C., J.A. Berry and H.A. Mooney. 1982. A portable system for measuring carbon dioxide and water vapour exchange of leaves. *Plant Cell Environ.* 5:179–186.
- García, D., J. Rodríguez, J.M. Sanz and J. Merino. 1998. Response of two populations of holm oak (*Quercus rotundifolia* Lam.) to sulfur dioxide. *Ecotox. Environ. Safe.* 40:42–48.
- Grill, D., M. Tausz and L.J. de Kok. 2001. Significance of glutathione in plant adaptation to the environment. Kluwer Academic Publisher, Dordrecht, 262 p.
- Hansen, L.D., R.S. Criddle, B.N. Smith and C. MacFarlane. 2002. Growth–maintenance component models are an inaccurate representation of plant respiration. *Crop Sci.* 42:659.
- Hesketh, J.D., D.N. Baker and W.G. Duncan. 1971. Simulation of growth and yield in cotton: respiration and the carbon balance. *Crop Sci.* 11:394–398.
- Lechowicz, M.J., L.E. Hellens and J.P. Simon. 1980. Latitudinal trends in the responses of growth respiration and maintenance respiration to temperature in the beach pea, *Lathyrus japonicus*. *Can. J. Bot.* 58:1521–1524.
- Lorenzini, G., S. Stringari and C. Nali. 2002. The absence of cross tolerance between ozone and paraquat: the case *Conyza bonariensis*. *Phyton* 42:89–96.
- Lusk, C.H. and P.B. Reich. 2000. Relationships of leaf dark respiration with light environment and tissue nitrogen content in juveniles of 11 cold-temperate tree species. *Oecologia* 123:318–329.
- Mariko, S. and H. Koizumi. 1993. Respiration for maintenance and growth in *Reynoutria japonica* ecotypes from different altitudes on Mt Fuji. *Ecol. Res.* 8:241–246.
- Martínez, F., Y.O. Lazo, R.M. Fernández-Galiano and J. Merino. 2002a. Chemical composition and construction cost for roots of Mediterranean trees, shrubs species and grasslands communities. *Plant Cell Environ.* 25:601–608.

- Martínez, F., Y.O. Lazo, J.M. Fernández-Galiano and J. Merino. 2002b. Root respiration and associated costs in deciduous and evergreen species of *Quercus*. *Plant Cell Environ.* 25:1271–1278.
- Mooney, H.A. 1963. Physiological ecology of coastal, subalpine, and alpine populations of *Polygonum bistortoides*. *Ecology* 44: 812–816.
- Ögren, E. 2000. Maintenance respiration correlates with sugar but not with nitrogen concentration in dormant plants. *Physiol. Plant.* 108: 295–299.
- Oleksyn, J., J. Modrzyński, M.G. Tjoelker, R. Zytowskiak, P.B. Reich and P. Karolewski. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Funct. Ecol.* 12: 573–590.
- Penning de Vries, F.W.T. 1975. The cost of maintenance processes in plant cells. *Ann. Bot.* 39:77–92.
- Penning de Vries, F.W.T., A.H.M. Brunsting and H.H. van Laar. 1974. Products, requirements and efficiency of biosynthesis: a quantitative approach. *J. Theor. Biol.* 45:339–377.
- Reich, P.B., M.G. Tjoelker, J.L. Machado and J. Oleksyn. 2006. Universal scaling of respiratory metabolism size and nitrogen in plants. *Nature* 439:457–461.
- Ryan, M.G. 1995. Foliar maintenance respiration of sub-alpine and boreal trees and shrubs in relation to nitrogen content. *Plant Cell Environ.* 18:765–772.
- Ryan, M.G., R.M. Hubbard, S. Pongracic, R.J. Raison and R.E. McMurtrie. 1996. Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree Physiol.* 16:333–343.
- Thornley, J.H.M. and M.G.R. Cannell. 2000. Modelling the components of plant respiration: representation and realism. *Ann. Bot.* 85: 55–67.
- Tjoelker, M.G., J. Oleksyn and P.B. Reich. 1999. Acclimation of respiration to temperature and CO<sub>2</sub> in seedlings of boreal tree species in relation to plant size and relative growth rate. *Global Change Biol.* 49:679–691.
- Villar, R. and J. Merino. 2001. Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. *New Phytol.* 151:213–226.
- Villar, R., J. Ruiz-Robledo, Y. de Jong and H. Poorter. 2006. Differences in construction costs and chemical composition between deciduous and evergreen woody species are small as compared to differences among families. *Plant Cell Environ.* 29:1629–1643.
- Vose, J.M. and M.G. Ryan. 2002. Seasonal respiration of foliage, fine roots, and woody tissues in relation to growth, tissue N, and photosynthesis. *Global Change Biol.* 8:182–193.
- Wright, I.J., P.B. Reich, M. Westoby et al. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Wright, I.J., B.R. Peter, O.K. Atkin, C.H. Lusk, M.G. Tjoelker and M. Westoby. 2006. Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. *New Phytol.* 169:309–319.
- Xu, C.Y. and K.L. Griffin. 2006. Seasonal variation in the temperature response of leaf respiration in *Quercus rubra*: foliage respiration and leaf properties. *Funct. Ecol.* 20:778–789.
- Zar, J.H. 1999. *Biostatistical analysis*, 4th Edn. Prentice-Hall, New Jersey, 663 p.
- Zaragoza-Castells, J., D. Sánchez-Gómez, F. Valladares, V. Hurry and O.K. Atkin. 2007. Does growth irradiance affect temperature dependence and thermal acclimation of leaf respiration? Insights from a Mediterranean tree with long-lived leaves. *Plant Cell Environ.* 30:820–833.