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Morphological and Physiological Response of Two Populations of *Quercus ilex* L. to SO₂ Fumigation

By

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Summary

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Quercus ilex L., is the predominant evergreen sclerophyllous tree in the Mediterranean landscape of the Iberian Peninsula. Fruit acorns were collected in two populations located in the center (southern Spain) and at the northern border (northern Spain; a distance of 800 km) of the distribution area of *Quercus ilex*. One-month-old potted plants were grown for 130 days to a high SO₂ concentration (0.23 ppm, 14 h d⁻¹) under controlled climate conditions. Both northern and southern plants underwent a significant decrease in growth rate as a consequence of the treatment. Even so, plants appear to be quite resistant to SO₂ compared with either more temperate or more productive species. The southern population was more sensitive to the treatment, as reflected by the bigger decrease in both growth and photosynthetic rates. Differences in resistance appear to be related to the biogeographic origin of the populations studied, which underlines the importance of biogeographic aspects in studies of resistance to air pollutants.

Introduction

In the last years, much attention has been given to analyzing the response of species to of air pollutants (WEIGEL & al. 1990, SANDERMANS & al. 1997). There are fewer studies on the response pattern in populations of the same species originating from different habitats, especially concerning their geographic location. However, knowing the response to an air pollutant of a species throughout its biogeographical area of distribution is mandatory if we want to predict the effects of that particular pollutant on the persistence of that species.

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The holm oak (*Quercus ilex* L.) is the predominant evergreen tree species in the Mediterranean landscapes of the Iberian Peninsula. It is found both as conserved populations, forming relatively dense woodlands, and as scattered individuals on grassland (savanna; locally known as "dehesa"). It extends from Southern Morocco (latitude 30° 40' N) to the north of the Iberian Peninsula (latitude 42° 30' N), where it is substituted by deciduous formations typical of the temperate European climate areas (*Quercus pyrenaica*, *Castanea sativa*, and *Fagus sylvatica*).

The aim of this study was to compare the sensitivity to SO₂ of individuals from two populations occupying distinct biogeographic locations. The morphological and physiological responses to fumigation with SO₂ of individuals from a population in northern Spain, located at the northern boundary of the distribution area of this species, were compared with those of individuals of a population in southern Spain. It is hypothesized that individuals originating from populations at the boundary of their distribution area should be more resistant to the effect of SO₂ because, in such environments, the species would be selected to resist multiple, and generally more intense, levels of stress (PARSONS 1993).

Material and Methods

Fruits of *Quercus ilex* were collected at two localities of the Iberian Peninsula (northern 42° 22' N, and southern 37° 58' N) (Fig. 1). The northern location corresponds to the northern boundary of the distribution area of this species. Acorns were sown in pots with a substrate of sand and vermiculite (1:1). On germination, the seedlings were kept in a greenhouse (45 days) until reaching 10 cm in height. From each population 90 seedlings were selected at random, and 60 were put into two indoor growth and fumigation chambers. The remaining 30 were used to estimate the mean dry weight (weight before treatment, WBT) of the individuals for each population.

Seedlings were irrigated daily with 100 % Hoagland's solution. Experimental conditions: photoperiod 14 h (PPFD = 312 $\mu\text{mol m}^{-2} \text{s}^{-1}$); day/night temperature, 24/18 °C; air relative humidity during the day was about 30-35 %. The air inside the chambers was exchanged approximately once per minute. The fumigation chamber had an injection device connected to pressurized SO₂. An automatic pumping system enabled the sampling of the plant's atmosphere inside the fumigation chamber and the almost continuous monitoring of the SO₂ concentration, using an analyzer (Model-4108, Dasibi, Glendale, CA, USA). The SO₂ concentration in the fumigation chamber atmosphere was kept at 0.23 ppm (v v⁻¹). The fumigation time was 14 h daily, coinciding with the period of illumination.

The experiment lasted 130 days. Each seedling was then separated into stem, leaf, and root fractions, oven dried at 80 °C for 48 h, and weighted (weight after treatment, WAT). The relative growth rate (RGR, $\text{mg g}^{-1} \text{d}^{-1}$) of each individual was calculated from WBT (the same for all individuals of a population) and from the WAT of the individual. The ratios LWR (leaf weight ratio, weight of leaves to plant weight), LAR (leaf area ratio, total surface of leaves to plant weight), SLW (specific leaf weight, leaf weight to leaf surface), and S/R (shoot to root weight) were also calculated. The values determined for each individual were used to calculate the mean values for each population studied.

Leaf photosynthetic rate (A_n), stomatal conductance (g_s) and CO₂ internal concentration (C_i) were estimated using an open gas exchange system (Model LCA-2; ADC, Hoddesdon, UK), under the same conditions of temperature, relative humidity, and light intensity as those of cultivation, but in the absence of SO₂.

Respiration rates (total (R_t) and maintenance (R_m)) were estimated between days 93 and 128, counting from the beginning of the treatment. Only intact leaves in the growth phase were

considered. For the estimate of total respiration rate, the CO_2 exchange in darkness and at 20°C was determined for all the selected leaves. The determinations were made using an open gas exchange system (Armstrong, Palo Alto, CA). The plants in which the determinations were made were kept in darkness to avoid the effect of carbohydrate level on respiration (VILLAR & al. 1995). After determination, the leaf was separated from the plant, dried, and weighed. The respiration rate was expressed as $\text{mg CO}_2 \text{ g}^{-1} \text{ d}^{-1}$.

The R_m of the leaves was estimated in accordance with the linear model of HESKETH & al. 1971 and the approach of KIMURA & al. 1978. It consists of regression (linear model) of the individual leaf specific respiration rate (SRR, $\text{mg CO}_2 \text{ g}^{-1} \text{ d}^{-1}$) in a set of leaves, on their respective specific growth rates (SGR, $\text{mg g}^{-1} \text{ d}^{-1}$). Extrapolation of the SRR (dependent variable) to zero SGR (independent variable) gives the respiration rate in the absence of growth; that is, the R_m (see AMTHOR 1989). The SGR of each leaf was determined from the increase in leaf surface in a period of 50 h and from SLW. The analysis of CO_2 evolution for the calculation of SRR was made in the same leaves and at the same time as the determination of the growth rate for the calculation of SGR.

Results

Fig. 2 shows the weights reached at the end of the experiment by the different fractions of the plants from the southern and northern populations. Southern individuals showed higher biomass in the different plant fractions, compared with northern individuals. The effect of the SO_2 treatment was similar for the two populations and resulted in a significant ($P < 0.05$) decrease in the final weight of the different fractions studied. The effect was stronger in the southern population, with decreases between 22 % and 36 % with respect to the control. In the northern population, the decreases were about 10 %, except for root weight, where the effect of the treatment was zero (Fig. 2). Northern individuals showed lower RGR (≈ 40 %) compared to southern individuals (17.3 and $27.6 \text{ mg g}^{-1} \text{ d}^{-1}$ respectively). The decrease in the weight of the fractions by the treatment resulted in a significant decrease ($P < 0.05$) in RGR (Fig. 3). In the northern population, RGR decreased from 17.3 to $16.0 \text{ mg g}^{-1} \text{ d}^{-1}$ (≈ 8 %) and, in the southern population, from 27.6 to $18.5 \text{ mg g}^{-1} \text{ d}^{-1}$ (≈ 33 %).

Individuals of the two populations have a different morphology, because all indices considered in the study were significantly different ($P < 0.05$), except for the SLW (Table 1). The northern population allocated more biomass to aerial structures (greater LWR, S/R, and LAR) than the southern one. SO_2 treatment did not significantly affect the values of most of the indices studied (LWR, LAR, S/R), suggesting that in each population the morphology of the plants is tightly controlled and, consequently, not altered by the effect of the SO_2 concentration considered in the present study. Even though not significant ($P > 0.05$), both the leaf area per individual (LA) and SLW changed as a consequence of the treatment. In the southern population LA decreased after fumigation (117 ± 15 and $856 \pm 13 \text{ cm}^2$ in control and fumigated plants, respectively), while fumigation had no effect on LA of the individuals in the northern population (82 ± 15 and $83 \pm 12 \text{ cm}^2$ for fumigated- and control plants, respectively). Fumigation decreased SLW in individuals of the northern population (from 170 ± 3 to $148 \pm 3 \text{ g m}^{-2}$), but fumigation

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had no effect on this parameter in plants from the southern population. No external signs of injury were observed in the leaves of the fumigated plants.

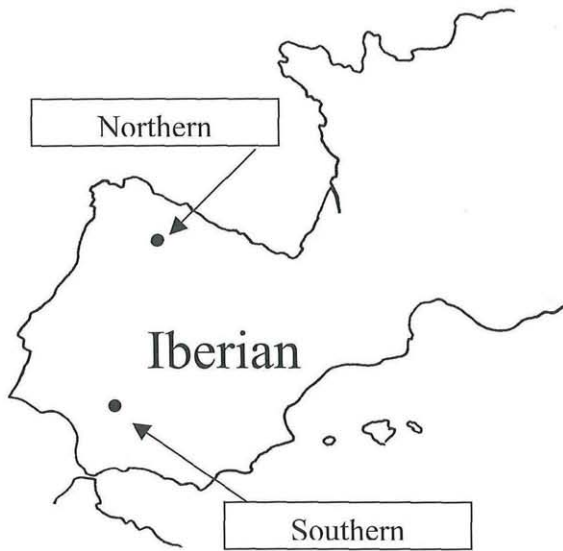


Fig. 1. Location of the populations studied at the Iberian Peninsula.

Northern and southern populations had an identical A_n (10.8 and 10.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ respectively, Table 2). Similar results were found for g_s (82 and 93 $\text{mmol m}^{-2} \text{ s}^{-1}$ respectively). As a result of the treatment, both g_s and A_n decreased significantly in both populations ($P < 0.05$) (Table 2). The decrease was less marked in the seedlings of the northern population ($\approx 30\%$ with respect to the control) than in those of the southern one ($\approx 65\%$). It should be noted that, as a consequence of the treatment, the average for g_s of the northern population was about twice as high (53.2 $\text{mmol m}^{-2} \text{ s}^{-1}$) as that estimated for the southern one (28.5 $\text{mmol m}^{-2} \text{ s}^{-1}$). There were no significant differences between populations either in the slopes of the photosynthesis-conductance curves or in the internal CO_2 concentrations. In addition, these variables did not differ between the control plants and those subjected to fumigation in either of the populations studied (Table 2). The R_i of the control plants was about 15% higher in the seedling leaves of the northern population than in those of the southern one ($P < 0.05$). Besides, the R_m of the leaves of the northern population was significantly greater ($\approx 27\%$, Table 2). The treatment resulted in a significant ($P < 0.05$) decrease ($\approx 15\text{--}31\%$) in R_i in both populations. The R_m also decreased significantly ($P < 0.05$) in the two populations to the same extent (23–25%), although its absolute value remained higher (about 28%) in the northern population.

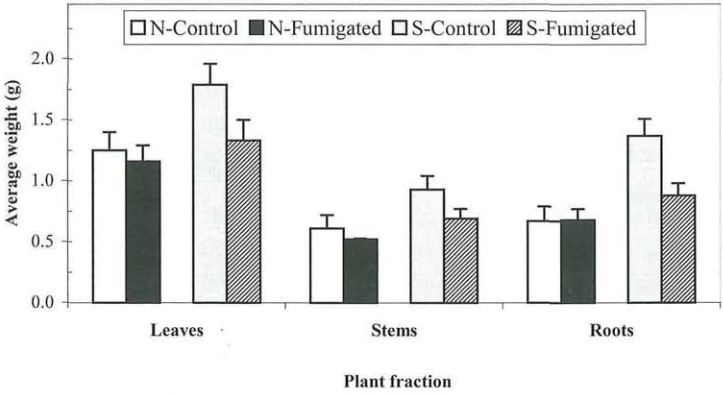


Fig. 2. Average weight of the different plant fractions (leaves, stem and roots) considered in both, northern (N) and southern (S) populations $\pm$ SD.

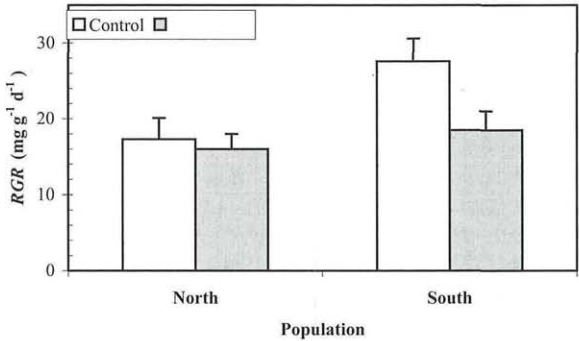


Fig. 3. Relative growth rate (RGR) of the two populations $\pm$ SD.

Discussion

The results indicate that the seedlings of the southern population have a higher RGR than those of the population from the north. Considering that A_n is virtually identical in the two populations, this result might appear as surprising, because the northern population invests more energy in aerial parts; that is, in the energy-gathering fraction of plant biomass. The higher R_m of the leaves of individuals in this population may explain their lower RGR. Maintenance respiration represents the energy cost associated with maintenance of structures and gradients. Despite its instantaneous value can be considered small (Table 2), integrated throughout the life of an organ, it can equal, or even surpass, the other leaf costs (MERINO & al. 1982, VILLAR & MERINO 2001). In fact, R_m is frequently correlated negatively with growth (AMTHOR 1989, TAYLOR 1989). The northern population's

greater investment in maintenance could offset its greater investment in aerial biomass, resulting in the lower RGR found in this population.

Table 1. Average values $\pm$ SD for the biomass allocation parameters in the two populations considered. For abbreviations see the text.

	LWR	LAR	S/R	SLW	n
Northern population					
Control	0.51 $\pm$ 0.02	30.2 $\pm$ 1.0	3.3 $\pm$ 0.4	169.9 $\pm$ 2.5	17
Fumigated	0.50 $\pm$ 0.02	33.5 $\pm$ 1.3	2.7 $\pm$ 0.3	147.6 $\pm$ 3.0	12
Effect of treatment	ns	ns	ns	ns	
Southern population					
Control	0.44 $\pm$ 0.01	27.0 $\pm$ 0.8	2.1 $\pm$ 0.1	164.0 $\pm$ 5.3	24
Fumigated	0.45 $\pm$ 0.02	27.4 $\pm$ 1.3	2.4 $\pm$ 0.2	164.0 $\pm$ 4.1	18
Effect of treatment	ns	ns	ns	ns	
Interpopulation differences	*	*	*	ns	

* Statistical significant difference at $P < 0.05$; ns, no significant difference.

Table 2. Average values $\pm$ SD for the physiological variables in the two populations considered. For abbreviations see the text.

	An	gs	Rt	Rm	Ci	Slope ^a	n
Northern population							
Control	10.8 $\pm$ 0.9	82 $\pm$ 8	77 $\pm$ 6	45 $\pm$ 9	292 $\pm$ 8	0.21 $\pm$ 0.01	24
Fumigated	7.6 $\pm$ 0.6	53 $\pm$ 5	58 $\pm$ 5	34 $\pm$ 8	282 $\pm$ 8	0.24 $\pm$ 0.01	24
Effect of treatment	*	*	*	*	ns	ns	
Southern population							
Control	10.5 $\pm$ 0.8	93 $\pm$ 8	66 $\pm$ 5	33 $\pm$ 9	309 $\pm$ 6	0.19 $\pm$ 0.01	24
Fumigated	3.9 $\pm$ 0.4	29 $\pm$ 3	57 $\pm$ 4	25 $\pm$ 11	294 $\pm$ 9	0.23 $\pm$ 0.01	24
Effect of treatment	*	*	*	*	ns	ns	
Inter-population differences	Ns	ns	*	*	ns	ns	

* Statistical significant difference at $P < 0.05$; ns, no significant difference.

^a Slope of the A_n/g_s curves (mmol mol^{-1}).

One of the most noteworthy effects of the SO_2 treatment was its almost total incapacity to alter the individual's architecture, suggesting that the arboreal nature of the species studied makes it less plastic than that of the herbaceous species studied by other authors (SCHLICHTING 1986, MOONEY & WINNER 1991). It should be noted that, in the northern population, the fumigated individuals keep the same LA as that of the control seedlings in spite of the significant decrease in leaf weight, which was the direct consequence of the decrease in SLW in response to

SO₂. In both populations RGR decreased as a consequence of the fumigation treatment, but the decrease was smaller than expected if considering the decrease in A_n , which could be explained by the observed decrease in R_m ($\approx 24\%$). Additionally, in the northern population, the observed smaller effect of the treatment on RGR appears to be result of compensatory processes involving LWR, SLW and LA, similar to those described in other SO₂ fumigation studies (MOONEY & WINNER 1991).

The mean RGR in the fumigated plants was similar in both populations, despite of both the lower value reached by A_n as a result of the fumigation in the southern population and the lower contribution of the aerial parts to the total weight (S/R) of the fumigated plants in this population, and to the compensatory mechanisms described earlier for the northern population. As discussed above for the non-fumigated plants, this apparent paradox can be explained by the difference in the maintenance cost between the two populations. This difference in maintenance cost is sustained under fumigation; maintenance respiration being some 28 % higher in the northern population, which possibly offsets the advantages of its relatively higher A_n and S/R ratio. The mean decrease in A_n induced by the treatment in the two populations was only 46 % with respect to the control. When the relatively high air SO₂ concentration, the length of the experiment, and the number of hours of fumigation per day are taken into account, *Q. ilex* can be classed as a very resistant species. Above all in comparison with the results published for other species in which the decrease under similar conditions can reach more than 80 % of its initial value (BARTON & al. 1980). The low g_s observed in control plants could explain, at least partly, the high resistance to fumigation in this species. Stomatal conductance is one of the main characteristics determining a species' resistance to air pollution (REICH & AMUDSON 1985, SANDERMANN & al. 1997).

There was a non-significant change in the slope of the photosynthesis-to-conductance curves by the effect of the treatment, and the maintenance of the initial values of the internal concentration of CO₂ in the fumigated plants of the two populations. This suggests that the decrease observed in A_n was basically a consequence of damage to the mesophyll rather than a result of stomatal closure as a direct effect of the treatment. It should be noted that northern population individuals have more resistant mesophyll than southern ones since they were able to keep higher g_s (and consequently, higher A_n rates) under fumigation. Because of their generally low g_s (KÖRNER 1994, KELLIHER & al. 1995) and the occurrence of frequent stomatal closure associated with water shortage (MARTINEZ-FERRI & al. 2000), Mediterranean species appear to have evolved defense mechanisms. These mechanisms may help to minimize the effects of molecular oxidative species generated under the (very common) situations of high light intensity and low substrate (CO₂) availability deriving from stomatal closure (FARIA & al. 1996). Besides, it should be noted that free radicals are also generated as a result of the primary reactions to SO₂ inside the leaf cells (PELL & DAN 1991). Therefore, if resistance to diverse stresses involves common underlying biochemical mechanisms (FANGMEIER & al. 1994, GRILL & al. 2001), such mechanisms could minimize the effect of SO₂ on the leaf mesophyll. In fact, some studies in crop plants have

demonstrated that resistance to SO_2 appears to be strongly related to free radical scavenging enzymes and endogenous antioxidant compounds (LEE & al. 1992).

In conclusion, *Q. ilex* and possibly many other Mediterranean woody evergreen species, could be considered pre-adapted to SO_2 pollution, given their inherently low gs as well as their low mesophyll sensitivity because of the presence of biochemical defensive mechanisms. Northern population individuals are more resistant (have a less-sensitive mesophyll) than the southern ones. Also, they appear to be more plastic because they are able to change their SLW in response to the treatment. It is difficult to explain the different resistance of the two populations to the treatment with SO_2 . The basic determinants of this difference in sensitivity might be related to the biogeographic origin of the populations. PARSONS 1990 has postulated the existence of strong relationships between biogeographic location resistance to stress, and the use that individuals make of energy. Studies carried out in animal species showed that, owing to the higher overall levels of stress in populations originating from the boundaries of their area of distribution, evolution seems to have favored more abundant defensive biochemical mechanisms in these populations, resulting in inherently more resistant individuals. The synthesis and operation of greater (and almost certainly more complex) defensive endowments mean a higher maintenance cost and thus a higher maintenance respiration rate (PARSONS 1993). The higher maintenance cost results in a lower availability of energy for growth processes and thus in lower growth rates in the individuals originating from populations at the area boundaries.

The syndrome described above matched very well the results obtained in the present study (low sensitivity of the photosynthetic process, higher maintenance cost, and lower growth rates in the northern populations). It emphasizes the need to consider the biogeographic origin of the populations in studies of resistance to air pollutants.

A c k n o w l e d g m e n t s

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