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# Chapter 21

# Effects of elevated carbon dioxide and acidic rain on the growth of holm oak

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**Abstract**

In order to study the interactive effects of elevated atmospheric $CO_2$ and acid rain on an evergreen oak, holm oak (*Quercus ilex* L.) (which is the keystone tree species in the Mediterranean environment) potted seedlings were grown for 90 days at two $CO_2$ concentrations, ambient and $+400$ µmol mol$^{-1}$. Half of each group was sprayed once a week with deionized (pH 5.6) or acidified (pH 2.5) water. Elevated $CO_2$ enhanced growth: shoot and leaf sprouting, shoot length, total leaf area, total and individual leaf mass, stem, and fine root mass were increased. Acid rain increased leaf and shoot turnover by stimulating both abscission and sprouting and, because abscission was more common than sprouting, the result was growth inhibition. The trend of these results was visible after 30–40 days. Total leaf area was increased by the increase in leaf number at elevated $CO_2$, and decreased by the reduction in leaf size in the acid treatments. Elevated $CO_2$ favored biomass partitioning to stems, whereas acid rain did not modify allocation. Root-to-shoot ratio was not significantly affected.

Despite the elevated $CO_2$-enhanced growth of acid-stressed holm oak seedlings, significant $CO_2 \times$ pH interactions were recorded only in cases where the effect of acidity was null (biomass allocation to stem) or positive (shoot and leaf sprouting). Thus, we conclude that short-term $CO_2$ enrichment did not alleviate the negative effect of acid rain on holm oak growth and that a strong acid load may inhibit the $CO_2$-promoted biomass partitioning to stems.

## 1. Introduction

Atmospheric carbon dioxide ($CO_2$) concentration is increasing at a rate of about 1.5 µmol mol$^{-1}$ yearly (Watson et al., 1990) and is predicted to reach

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600–800 µmol $\text{mol}^{-1}$ by the end of the 21st century (Conway et al., 1988). This global phenomenon may interact locally with the deposition of acidifying substances, whose critical loads (the levels of deposition above which long-term harmful effects can be expected) are exceeded in about 10% of the European area (European Environment Agency, 1998).

Growth enhancement under elevated $CO_2$ is a general response for young trees (Ceulemans and Mousseau, 1994). Simulated acid rain may induce either an increase or a reduction in growth or may have no effect on growth (Lee and Weber, 1979; Jacobsen et al., 1990; Neufeld et al., 1985). Possible synergistic effects of acid rain and elevated $CO_2$ on aboveground tree growth have not yet been investigated.

We explored their short-term effects on growth, anatomy, and biomass partitioning of holm oak (*Quercus ilex* L.) seedlings. Holm oak is a drought- and shade-tolerant evergreen broadleaf tree, which forms climax forest communities over large areas of the Mediterranean basin. Holm oak biomass and metabolism have been shown to be stimulated in $CO_2$-enriched environments (Chaves et al., 1995; Hättenschwiler et al., 1997a, 1997b; Tognetti et al., 1998; Tognetti and Johnson, 1999). Holm oak root and mycorrhizal response to elevated $CO_2$ and acid rain has been the object of a previous study (Puppi et al., 1992). Other responses to acid depositions in holm oak are unknown. The object of this study was to determine if a strong acid load may inhibit the growth stimulation induced in holm oak by doubling atmospheric $CO_2$.

## 2. Material and methods

One hundred and twenty 2-year-old holm oak seedlings, grown in pots (2.5 l, peat : vermiculite : nursery soil $= 70:15:15$) in the same nursery and all the same size, were placed in two controlled chambers (LABCO Mod. CT15, 25/18 °C day/night temperature, 10-hour photoperiod, 250 µE $\text{m}^{-2}\,\text{s}^{-1}$ PPFD, 60% RH, 0.5 $\text{m}\,\text{s}^{-1}$ air flow), irrigated daily with water and once a week with Hoagland solution to attain field capacity, and rotated once a week to avoid position effects. The growing conditions were intended to simulate a Mediterranean spring under a closed canopy, as holm oak is a shade-tolerant species (Valladares et al., 2000).

Carbon dioxide was maintained at two constant concentrations, ambient ($\approx$ 360 µmol $\text{mol}^{-1}$) and ambient $+$ 400 µmol $\text{mol}^{-1}$, by an electronic flowmeter and a gas analyzer (CIM-1 and EGM-1, PP Systems, UK). In each controlled chamber, half of each $CO_2$ group (30 seedlings each) was sprayed with deionized (pH 5.6) or acidified (pH 2.5, $H_2SO_4 : HNO_3 = 2:1$ in equivalents) water through stainless steel nozzles at a 3 mm $\text{min}^{-1}$ rate for 15 min $\text{week}^{-1}$

in the same day each week, until dripping point. The treatments (control, acid, elevated $CO_2$, combined) lasted 90 days.

After labelling, the number and length of shoots and number of leaves were recorded every 4–9 days. At harvest, leaf area was measured by LI-COR 3100, and dry weights of leaves, stems, and roots were recorded after reaching a constant value in an oven. The root system was divided into coarse (diameter $>$ 2 mm) and fine (diameter $<$ 2 mm) roots. New leaf area/dry mass (specific leaf area, SLA), new leaf area/new shoot dry mass (leaf area ratio, LAR), ratios of new leaf (leaf weight ratio, LWR), new stem (stem weight ratio, SWR), and fine root (root shoot ratio, R/S) dry mass to new shoot dry mass were calculated. Cross-sections of leaves and stems sprouted after 55–75 days of treatment, were sampled from the central part of mature, fully expanded leaves, and from stem segments 1.5 cm below the shoot apex. There was one sample per seedling, ten samples per treatment. At harvest, ten 1-cm long segments were removed 2 cm below the fine root apex from each of three seedlings per treatment. After fixation in formalin–acetic acid–alcohol and embedding in butyl and methyl methacrylate (7 : 3), samples were sectioned into 2 µm segments by ultramicrotome Reichert om U3 and stained with periodic acid-Schiff's reaction or Lugol (Jensen, 1962) for starch grains and with toluidine blue O (Trump et al., 1961) for anatomical measurements. Because the pith in the stem was in the shape of a five-pointed star, the mean diameter for each sample was calculated as mean length of the lines through the star points. Six cross-sectional measurements were taken for each leaf and each root segment. Spongy cell area and size, and starch grain area, with respect to cell area were quantified by the WinDias image analysis system (1.5 Delta-T Devices) on one photograph per cross-section.

Data were analyzed by two-way ANOVA ($CO_2 \times$ pH). The statistical unit was the individual pot ($n = 30$, except for fine root anatomy where $n = 3$). Data expressed in percentages (variation in shoot and leaf final number with respect to the initial one, spongy cell area with respect to the spongy mesophyll area, starch area with respect to the cell area) were previously arc sine transformed.

## 3. Results

### *3.1. Growth*

Elevated $CO_2$ stimulated shoot and leaf sprouting (Fig. 1(A), (D)), new shoot elongation (Fig. 1(B)), and leaf sprouting in each shoot (Fig. 1(C)). Acid rain stimulated shoot and leaf sprouting (Fig. 1(A), (D)) and inhibited shoot length and leaf sprouting in each shoot (Fig. 1(B), (C)). A significant increase

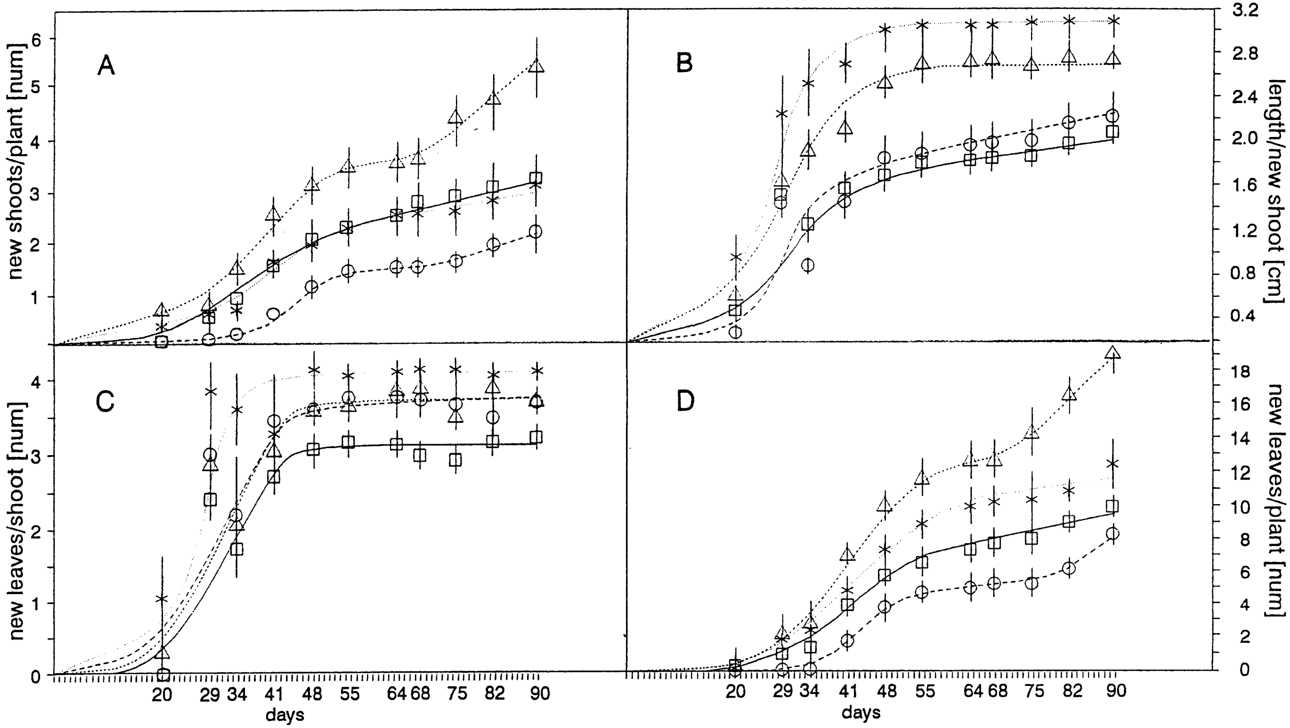

*Figure 1.* Shoot number (A) and length (B), leaf number per shoot (C) and per seedling (D) during the 90-day test period for the control (O, ambient $CO_2$ + pH 5.6 rain), acid (□, ambient $CO_2$ + pH 2.5 rain), elevated $CO_2$ (×, +400 µmol $mol^{-1}$ $CO_2$ + pH 5.6 rain), and combined (Δ, +400 µmol $mol^{-1}$ $CO_2$ + pH 2.5 rain) treatment. Two-way analysis of variance was applied to the final data, in the box: $\ast$ $p \leqslant 0.05$, $\circ$ $p \leqslant 0.1$, ns: $p > 0.1$.

| | ANOVA | | |
|---|---|---|---|
| | $CO_2$ | pH | $CO_2 \times$ pH |
| A | ○ | ○ | ○ |
| B | ∗ | ○ | ns |
| C | ○ | ○ | ns |
| D | ○ | ○ | ○ |

*Figure 1.* (*Continued*)

*Table 1.* Morphobiometric characteristics at the harvest. Increase/decrease in shoots and leaves were calculated with respect to the initial number

| | Treatments | | | | ANOVA[a] | | |
|---|---|---|---|---|---|---|---|
| | Control | Acid | Elevated $CO_2$ | Combined | $CO_2$ | pH | $CO_2 \times$ pH |
| Increase/decrease in shoots [%] | 8.5 | −1.9 | 25.3 | 0.4 | ∗ | ∗ | ns |
| Fallen shoots per plant [num] | 1.7 | 3.4 | 2.3 | 5.5 | ○ | ∗ | ns |
| Increase/decrease in leaves [%] | −2.5 | −22.2 | 17.2 | 8.8 | ∗ | ∗ | ns |
| Abscissed leaves per plant [num] | 9.8 | 24.5 | 6.5 | 17.6 | ○ | ∗ | ns |
| Leaf area per plant [$cm^2$] | 81.2 | 60.8 | 105.6 | 100.0 | ∗ | ○ | ns |
| Leaf area per leaf [$cm^2$] | 9.9 | 6.0 | 8.5 | 5.2 | ○ | ○ | ns |

[a] ∗ $p \leqslant 0.05$, ○ $p \leqslant 0.1$, ns: $p > 0.1$.

in shoot and leaf sprouting was recorded in the $CO_2 \times$ pH treatment. Such results were significant ($p < 0.05$) as early as 30–40 days after beginning the treatment (Fig. 1). After 75 days, seedlings began a second growth flush (Fig. 1(A), (D)).

Elevated $CO_2$ increased final shoot and leaf number despite increased abscission, and increased leaf area despite decreasing leaf size (Table 1). Acid rain favored both shoot and leaf number and abscission, and also inhibited leaf area and size (Table 1). Leaf area showed no significant $CO_2 \times$ pH interaction.

### ***3.2. Biomass***

Elevated $CO_2$ increased dry weight of the total leaves, single leaf, stems, new shoots, and fine roots (Table 2). Coarse root biomass was not influenced by any

*Table 2.* Dry weight (dw) for roots and shoots sprouted during the test period [mg]

| | Treatments | | | | ANOVA[a] | | |
|---|---|---|---|---|---|---|---|
| | Control | Acid | Elevated $CO_2$ | Combined | $CO_2$ | pH | $CO_2 \times$ pH |
| Leaf dw per plant | 565 | 440 | 889 | 915 | $*$ | ns | ns |
| Leaf dw per leaf | 69 | 44 | 72 | 48 | $\circ$ | $\circ$ | ns |
| Stem dw per plant | 121 | 100 | 297 | 222 | $*$ | $\circ$ | ns |
| Shoot dw per plant | 696 | 544 | 1189 | 1142 | $*$ | $\circ$ | ns |
| Fine root dw per plant | 338 | 520 | 774 | 740 | $\circ$ | ns | ns |
| Coarse root dw per plant | 4520 | 4115 | 3596 | 4772 | ns | ns | ns |

[a] $* \ p \leqslant 0.05$, $\circ \ p \leqslant 0.1$, ns: $p > 0.1$.

*Table 3.* Allocation parameters for fine roots and for shoots sprouted during the test period. SLA: Specific Leaf Area; LAR: Leaf Area Ratio; LWR: Leaf Weight Ratio; SWR: Stem Weight Ratio; R/S: fine Root/new Shoot dry weight

| | Treatments | | | | ANOVA[a] | | |
|---|---|---|---|---|---|---|---|
| | Control | Acid | Elevated $CO_2$ | Combined | $CO_2$ | pH | $CO_2 \times$ pH |
| SLA [$cm^2\,mg^{-1}$] | 0.142 | 0.138 | 0.119 | 0.109 | $\circ$ | ns | ns |
| LAR [$cm^2\,mg^{-1}$] | 0.118 | 0.113 | 0.090 | 0.088 | $\circ$ | ns | ns |
| LWR [$mg\,mg^{-1}$] | 0.823 | 0.816 | 0.758 | 0.814 | ns | ns | ns |
| SWR [$mg\,mg^{-1}$] | 0.174 | 0.184 | 0.250 | 0.194 | $\circ$ | ns | $\circ$ |
| R/S [$mg\,mg^{-1}$] | 0.486 | 0.956 | 0.651 | 0.648 | ns | ns | ns |

[a] $\circ \ p \leqslant 0.1$, ns: $p > 0.1$.

of the treatments. Acid rain reduced the dry weight of the single leaf, stems, and new shoots. Interactions between $CO_2$ and pH were not significant.

Specific leaf area and LAR were reduced and SWR was increased at elevated $CO_2$ (Table 3). Leaf weight ratio and R/S did not vary significantly. Acid rain did not change biomass allocation parameters. A significant $CO_2 \times$ pH interaction was found for SWR.

### *3.3. Anatomy*

Leaf tissue thickness did not vary (Table 4). The palisade was consistently composed of at least one layer, and more frequently two layers, of cells. At elevated $CO_2$, the shape of spongy cells was irregularly elongated, but did not vary in size, and a reduction in intercellular spaces was observed (Table 4). These results suggest that the number of spongy cells increased (data not shown). Starch was more abundant at elevated $CO_2$, particularly in the palisade cells (Table 4).

*Table 4.* Thickness of leaf tissues [μm], cell area in the spongy mesophyll [%], spongy cell size [$\mu m^2$], and area of starch grains in the palisade and spongy cells [%]

| | Treatments | | | | ANOVA[a] | | |
|---|---|---|---|---|---|---|---|
| | Control | Acid | Elevated $CO_2$ | Combined | $CO_2$ | pH | $CO_2 \times$ pH |
| Upper epidermis | 14 | 14 | 15 | 15 | ns | ns | ns |
| Palisade | 75 | 75 | 80 | 66 | ns | ns | ns |
| Spongy | 85 | 100 | 85 | 85 | ns | ns | ns |
| Lower epidermis | 10 | 11 | 10 | 11 | ns | ns | ns |
| Total thickness | 184 | 200 | 190 | 176 | ns | ns | ns |
| Cell area in the spongy mesophyll | 58 | 59 | 66 | 65 | $*$ | ns | ns |
| Spongy cell size | 201 | 200 | 254 | 227 | ns | ns | ns |
| Starch area in the palisade | 2.4 | 2.6 | 56.4 | 49.5 | $**$ | ns | ns |
| Starch area in the spongy mesophyll | 1.5 | 1.4 | 2.7 | 2.7 | $*$ | ns | ns |

[a] $**$ $p \leqslant 0.01$, $*$ $p \leqslant 0.05$, ns: $p > 0.1$.

*Table 5.* Thickness of stem tissues [μm] and area of starch grains in the cortex and pith cells [%]

| | Treatments | | | | ANOVA[a] | | |
|---|---|---|---|---|---|---|---|
| | Control | Acid | Elevated $CO_2$ | Combined | $CO_2$ | pH | $CO_2 \times$ pH |
| Periderm | 36 | 39 | 41 | 37 | ns | ns | ns |
| Cortex | 135 | 181 | 159 | 210 | ns | ns | ns |
| Sclerenchyma | 35 | 35 | 36 | 43 | ns | ns | ns |
| Phloem | 91 | 62 | 103 | 105 | $\circ$ | ns | ns |
| Xylem | 106 | 127 | 211 | 228 | $*$ | ns | ns |
| Pith | 492 | 614 | 594 | 612 | ns | ns | ns |
| Total ray | 919 | 1065 | 1150 | 1227 | $*$ | ns | ns |
| Starch area in the cortex | 0 | 0 | 0.5 | 0.6 | $*$ | ns | ns |
| Starch area in the pith | 0.14 | 0.15 | 11.3 | 10.9 | $**$ | ns | ns |

[a] $**$ $p \leqslant 0.01$, $*$ $p \leqslant 0.05$, $\circ$ $p \leqslant 0.1$, ns: $p > 0.1$.

Stem thickness was increased at elevated $CO_2$ because of the increase in phloem and xylem production (Table 5). Stems at elevated $CO_2$ showed a starch surplus in the cortex and especially in the pith (Table 5).

Fine root thickness and starch content did not vary significantly (data not shown). Effects of acid treatments and $CO_2 \times$ pH interactions were not significant.

## 4. Discussion

### *4.1. Overall growth*

Elevated $CO_2$ promoted shoot and leaf initiation, and shoot length. Growth enhancement under elevated $CO_2$ is a general response for young trees (Ceulemans and Mousseau, 1994), although no effect on height has been reported in *Picea glauca* or *Pinus radiata* (Brown and Higginbotham, 1986; Conroy et al., 1986) and a shoot growth reduction has been recorded for *Castanea sativa* (Mousseau and Enoch, 1989). A weakening in apical dominance and a greater number of side shoots under $CO_2$ enrichment have also been recorded on *Liquidambar styraciflua*, *Citrus aurantium*, and two *Populus* clones (Sionit et al., 1985; Idso et al., 1991; Ceulemans et al., 1995).

Acid rain promoted shoot initiation and inhibited leaf initiation—as shown by the variation in the number of new leaves per plant and per shoot—and shoot length. Simulated acid rain has been found to induce both an increase and a reduction in shoot elongation as well as a lack of variation in many conifers and deciduous broadleaves (Lee and Weber, 1979; Jacobsen et al., 1990; Neufeld et al., 1985). Nonetheless, stimulation of shoot and leaf sprouting by acid spray on epigeous parts confirmed the results of Winner et al. (1985), supporting the hypothesis that photosynthates were partitioned above all into portions under stress. However, acidity also promoted abscission, so that leaf and shoot turnover increased. Shoot abscission in the *Quercus* genus is a common phenomenon (Millington and Chaney, 1973), although it occurs mainly in autumn. Serious and unusual environmental stresses also promote the loss of plant parts (Kozlowski, 1973). Controls showed slight shoot and leaf abscission, even though the second growth flush indicated that chamber conditions were optimal for growth. Oak seedlings are known to show recurrent cyclic flushes which, if not synchronized, can induce excessive variability in samples (Norby and O'Neill, 1989). Periodic non-destructive measurements allowed us to compare the growth models of each plant, thus avoiding sampling errors. Furthermore, our treatments did not modify the growth flush synchrony (Fig. 1). Significant $CO_2 \times \mathrm{pH}$ interactions were recorded for total leaf and shoot sprouting when both elevated $CO_2$ and acid rain individually stimulated initiation. Thus, no inhibiting effect of acidity on $CO_2$-enhanced growth was detected.

### *4.2. Leaf area*

Elevated $CO_2$ increased total leaf area and decreased individual leaf area, i.e., leaf size. A previous investigation on holm oak response to $CO_2$ enrichment also promoted total leaf area, but leaf size did not change (Tognetti and Johnson, 1999). An increase in total leaf area has often been found under elevated

$CO_2$ concentrations in both conifers and deciduous broadleaves (Ceulemans and Mousseau, 1994; Janssens et al., 2000). The findings of the present study show that the increase in total leaf area in the elevated $CO_2$ treatment was associated with an increase in leaf number, indicating a strong morphogenic effect of $CO_2$ on leaf initiation, as found in *Liquidambar styraciflua* (Tolley and Strain, 1984), *Quercus petraea* (Guehl et al., 1994) and two *Populus* clones (Ceulemans et al., 1995). Leaf size may respond to an elevated $CO_2$ supply by increasing (Conroy et al., 1986; Koch et al., 1986; Gaudillère and Mousseau, 1989), not changing (Tolley and Strain, 1984; Radoglou and Jarvis, 1990) or decreasing (Mousseau and Enoch, 1989). A $CO_2$-induced decrease in leaf size contrasts with the enhancement in leaf cell expansion reported for some grassland herbs (Ferris and Taylor, 1994) and *Populus* clones (Radoglou and Jarvis, 1990; Gardner et al., 1995), and supports the hypothesis that leaf ontogenesis response to $CO_2$ is species dependent.

Acid rain reduced both total and individual leaf area. The reduction in total leaf area, also reported in *Brassica oleracea* (Caporn and Hutchinson, 1986), was related to the decreasing leaf size as the total number of leaves increased, demonstrating that acidity significantly influenced both shoot initiation and leaf ontogenesis.

Despite the ability of both $CO_2$ and acid rain to influence leaf initiation and ontogenesis in holm oak, no significant alleviating or inhibiting interaction was recorded for leaf area.

### *4.3. Leaf biomass and anatomy*

The increase in total leaf mass at elevated $CO_2$ was associated with a greater number of leaves and, in contrast to the above-mentioned decreasing leaf size, with an increase in individual leaf mass. As leaf thickness did not vary, the higher starch content and smaller intercellular spaces in the spongy mesophyll may have contributed to this increase. A greater starch content in leaves grown at elevated $CO_2$ has frequently been reported (Patterson and Flint, 1980; Cave et al., 1981; Wulff and Strain, 1982; Yelle et al., 1989), even for holm oak (Tognetti and Johnson, 1999). The increase in individual leaf mass was the result of increased cell division, which was sensitive to $CO_2$, whereas cell enlargement apparently was not. In *Populus* clones and *Phaseolus vulgaris*, more extensive and slightly decreasing intercellular air spaces were recorded (Radoglou and Jarvis, 1990, 1992, respectively), again suggesting a species-dependent response of leaf ontogenesis to $CO_2$. In the same species, elevated $CO_2$ increased leaf thickness, mainly as a result of larger mesophyll cells (Radoglou and Jarvis, 1990, 1992). In $C_3$ plants at high $CO_2$, Thomas and Harvey (1983) observed an increase in leaf thickness that was associated with a third layer of palisade cells. In our study, similarly to Hofstra and Hesketh

(1975) for *Glycine max* and Radoglou and Jarvis (1993) for *Vicia faba*, the number of palisade layers did not change with variation in $CO_2$.

An increase in total leaf mass has been found in all high $CO_2$ studies (e.g., Norby and O'Neill, 1989; O'Neill et al., 1987; Koch et al., 1986; Sionit et al., 1985), but exposure to simulated acid depositions has been found to result in no variation (Neufeld et al., 1985) or reduction (Evans and Lewin, 1980). The acid-induced decrease in total leaf mass observed in the present experiment was not significant as it was offset by the increase in leaf number. The reduction in individual leaf mass in the acid treatments was due to the decrease in individual leaf area, as no significant anatomical modification was found.

Interactions between $CO_2$ and pH were not significant, with acid rain reducing individual leaf mass to the same extent in seedlings raised at ambient and elevated $CO_2$. Acid rain did not change total leaf mass in either ambient or elevated $CO_2$ treatments.

### *4.4. Stem biomass and anatomy*

Stem mass at elevated $CO_2$ increased, similarly to Rogers et al. (1983), Higginbotham et al. (1985), Koch et al. (1986), O'Neill et al. (1987), Norby and O'Neill (1989), as a result of the stimulation in shoot elongation and phloem and xylem production, thus suggesting that $CO_2$ promoted activity in both apical meristems and vascular cambium. The starch surplus found in the cortex and pith at elevated $CO_2$ may have contributed to the increase in stem dry weight.

In accordance with Neufeld et al. (1985), acid rain decreased stem mass, as a result of the inhibition in shoot elongation rather than of anatomical modifications, thus suggesting that acidity depressed apical meristem activity and did not influence the vascular cambium.

Despite the influence of both $CO_2$ and acid rain on stem meristematic tissues, in the combined treatment there was no evidence that $CO_2$ enrichment afforded additional protection against stem biomass decrease induced by acidity.

### *4.5. Root biomass and anatomy*

Elevated $CO_2$ increased fine root biomass, as reported elsewhere for holm oak seedlings (Tognetti and Johnson, 1999) and in agreement with many studies recording substantial root growth at elevated $CO_2$ (Dahlman, 1993). No variation was found in coarse root dry weight, as expected, given that coarse roots developed during the 2 years preceding the experiment.

No significant differences in starch distribution and tissue thickness were observed, probably because of sampling artifacts due to the inability to perform direct observations on root growth and to collect roots at the same developmental stage.

Although Neufeld et al. (1985) and Lee et al. (1981) found that simulated acid depositions applied to foliage reduced root dry weight, fine root biomass in this experiment did not change. The increase in fine root biomass induced by elevated $CO_2$ under acid rain was similar to that induced by $CO_2$ under pH 5.6 rain.

### *4.6. Biomass partitioning*

Most experiments cited in Ceulemans and Mousseau (1994) and Janssens et al. (2000) reported an increase in root/shoot ratio, suggesting that $CO_2$ enrichment preferentially induces extra root storage. The lack of response observed in our experiment could be attributable to the small pot size (Arp, 1991) or to other environmental variables (Janssens et al., 2000). Another study using holm oak seedlings grown in one-quarter smaller pot volumes has found evidence of a preferential shift of biomass to belowground tissues in response to $CO_2$ enrichment (Tognetti and Johnson, 1999).

The decrease in LAR at elevated $CO_2$ observed in our experiment was considered a result of the reduction in SLA, as reported elsewhere for holm oak seedlings (Tognetti and Johnson, 1999). This suggests that foliage mass increased sufficiently to compensate for the reduction in leaf size. According to Tolley and Strain (1984), the decrease in SLA in plants grown at high $CO_2$ could be due to an increase in leaf thickness and/or quantity of starch. In our experiment, starch accumulation (in agreement with Tognetti and Johnson, 1999) was associated with greater cell density in the spongy cells, while leaf thickness remained constant.

The increase in SWR at high $CO_2$ and the lack of variation in LWR indicate that biomass allocation tended to favor the stems, as confirmed by the increase in stem thickness and shoot elongation.

Leaf sprouting was likely to be the most important carbon sink in the acid-treated seedlings. Abscission was also promoted, so that acid rain reduced shoot mass without apparent shifts in allocation. Studies on several deciduous broadleaves and conifers have also found no influence of acid depositions on dry matter partitioning (Tolley and Strain, 1984; Neufeld et al., 1985; Norby and O'Neill, 1989; Deans et al., 1990).

Acid rain reduced the $CO_2$-induced increase in SWR in our combined treatment, probably because of the reduction in stem length, given that no anatomical difference was detected. This was the only case in which the acid treatment inhibited a positive effect induced by the elevated $CO_2$.

## 5. Conclusions

Both elevated $CO_2$ and acid rain induced morphogenetic responses in holm oak, the effects of which are likely to be important in predicting stand productivity. Elevated $CO_2$ promoted the growth of holm oak seedlings, despite the fact that holm oak, like all sclerophyllous species, shows relatively low $CO_2$ exchange rates (Mooney, 1986). Acid mist accelerated leaf and shoot turnover by stimulating both abscission and sprouting, and inhibited the overall growth, as abscission was more common than sprouting.

Trees in natural stands may morphologically adjust to increasing $CO_2$ and reduce $CO_2$-induced initial growth stimulation. In fact, contrary to our results, mature holm oaks in naturally $CO_2$-enriched sites showed decreased branching and lower total leaf area (Hättenschwiler et al., 1997a). They also showed an increase in stem mass that was largely due to responses when trees were young (Hättenschwiler et al., 1997b) and confirms the increased biomass partitioning to stems reported here.

In contrast, acid rain reduced leaf and stem mass without apparent significant shifts in allocation. Together with stem and fine roots, leaf sprouting was one of the most important carbon sinks in the $CO_2$-treated seedlings, and was likely the most important one in the acid-treated seedlings, even if the increase in abscission concealed it.

Our study shows that increasing $CO_2$ concentrations enhanced the growth of acid-stressed holm oak seedlings. However, significant $CO_2 \times$ pH interactions were recorded only for the final number of shoots and leaves, and for biomass allocation to stems. In both cases, the effect of acidity was null or positive, and the effect of $CO_2$ was positive. Thus, no ameliorating effect of short-term $CO_2$ enrichment on negative responses induced by acid rain was detected, although an inhibiting effect of acidity on the $CO_2$-increased biomass partitioning to stems was noted.

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