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Responses of Antioxidative Systems to Drought Stress in Pedunculate Oak and Maritime Pine as Modulated by Elevated CO_2[1]

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The aim of the present study was to investigate the effects of an enhanced CO_2 concentration alone or in combination with drought stress on antioxidative systems of a deciduous (oak; *Quercus robur*) and an evergreen (pine; *Pinus pinaster*) tree species. The seedlings were grown for one season in a greenhouse in tunnels supplied with 350 or 700 μL L^{-1} CO_2. The experiment was repeated in a second year. Antioxidants, protective enzymes, soluble protein, and pigments showed considerable fluctuations in different years. Elevated CO_2 caused significant reductions in the activities of superoxide dismutases in both oak and pine. The activities of ascorbate peroxidase and catalase were also reduced in most cases. The activities of dehydroascorbate reductase, monodehydroascorbate radical reductase, glutathione reductase, and guaiacol peroxidase were affected little or not at all by elevated CO_2. When the trees were subjected to drought stress by withholding water, the activities of antioxidative enzymes decreased in leaves of pine and oak grown at ambient CO_2 and increased in plants grown at elevated CO_2 concentrations. The present results suggest that growth in elevated CO_2 might reduce oxidative stress to which leaf tissues are normally exposed and enhance metabolic flexibility to encounter increased stress by increases in antioxidative capacity.

Most predictions suggest that the current mean ambient CO_2 concentration of 355 μL L^{-1} will approximately double by the end of the next century (Roeckner, 1992). Increasing concentrations of CO_2 and of other greenhouse gases will result in an increase in mean temperature and cause changes in precipitation patterns (Roeckner, 1992). Climate models predict large-scale drought periods during summer for northern mid-latitudes (Roeckner, 1992). The availability of water is one of the most important factors determining vegetation diversity and plant productivity (Rochefort and Woodward, 1992). The effects of water deficits on plant performance and growth are mediated through decreases in stomatal conductance and photosynthesis and depend on the severity and duration of the drought period, the presence of further environmental constraints, and species-inherent characteristics (Chaves and Pereira, 1992). In C_3 plants, elevated atmospheric CO_2 concentrations can partially compensate for the negative effects of drought by increasing water use efficiency and by sustaining larger net CO_2 assimilation rates at reduced stomatal conductance in leaves of stressed plants (Chaves and Pereira, 1992).

The photosynthetic apparatus per se appears to be highly resistant to injury resulting from water deficits (Kaiser, 1987; Cornic et al., 1989; Quick et al., 1992). Conditions of limited water supply that result in stomatal closure and restricted CO_2 fixation rates require that plants dissipate excess light energy to avoid photooxidative destruction. Several mechanisms acting either to reduce the photochemical efficiency of photosystems, such as the xanthophyll cycle (Pfündel and Bilger, 1994), or to maintain a substantial electron flow despite a low CO_2 availability in the chloroplasts, such as photorespiration and the Mehler peroxidase reaction, have been implicated in providing protection of the photosynthetic apparatus from photooxidative damage (Foyer and Harbinson, 1994). It has also been proposed that molecular O_2, which normally serves as an electron acceptor at PSI at a low rate (Mehler reaction), might provide a valve for excess reductant (Elstner and Osswald, 1994). Both pathways, the Mehler reaction and photorespiration, implicate an increased production of potentially toxic O_2 species such as $O_2^{\cdot -}$ and H_2O_2.

In the chloroplasts, the primary product of the Mehler reaction, $O_2^{\cdot -}$, is rapidly scavenged by superoxide dismutases, yielding H_2O_2. Photorespiration also yields H_2O_2, namely when the primary product of the oxidative degradation of ribulose-1,5-bisP, glycolate, is oxidized to glyoxylate in the peroxisomes. To prevent oxidative damage, plants possess antioxidative systems composed of low molecular weight antioxidants such as ascorbate and glutathione and protective enzymes such as superoxide dismutases, catalases, and peroxidases (Alscher et al., 1991). Other components of this system, monodehydroascorbate radical reductase, dehydroascorbate reductase, and glutathione reductase, serve to maintain the antioxidants in their reduced functional state (Alscher et al., 1991). In several herbaceous plant species, antioxidative systems were induced in response to drought stress, suggesting that water deficits

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imposed an increased oxidative stress (Smirnoff, 1993). In leaves subjected to severe dehydration, the compensation of oxidative stress was apparently insufficient because increased concentrations of superoxide radicals and lipid peroxidation were observed in injured tissues (Price and Hendry, 1991; Quartacci and Navari-Izzo, 1992; Smirnoff, 1993). Field studies conducted in a natural stand of oak (*Quercus robur* and *Quercus petrea*) trees showed that oak was a drought-tolerant species because full photosynthetic capacity was maintained even after periods of severe dehydration, resulting in predawn leaf water potentials of less than −2.0 MPa (Epron and Dreyer, 1993a, 1993b). Therefore, it was concluded that oak leaves possess efficient protection mechanisms from photooxidative injury (Epron and Dreyer, 1993a). The nature of these protective systems was not investigated.

Pendunculate oak (*Q. robur*) and maritime pine (*Pinus pinaster*) cover large-scale forest areas in France. In a changing environment, the performance of these economically and ecologically important tree species is of particular interest, since the seedlings used in forest plantations to date are likely to experience the projected changes in environmental conditions within their lifetime. It has been observed that growth and biomass accumulation of pendunculate oak and maritime pine seedlings benefit significantly from elevated atmospheric CO_2 concentrations (Guehl et al., 1994; Seegmüller and Rennenberg, 1994; Picon et al., 1995). It is unknown to what extent the potentially damaging effects of drought-induced oxidative stress might be alleviated in an environment with elevated CO_2. Therefore, the goal of the present study was to investigate the response of photosynthesis and antioxidative systems in oak and pine seedlings subjected to drought stress under current and elevated atmospheric CO_2 concentrations.

MATERIALS AND METHODS

Growth of Plants, Drought Treatment, and Harvest

Acorns (*Quercus robur*, L. provenance Manoncourt) were collected in November 1992 in a parent stand close to Nancy in Lorraine (France). The acorns were washed for 3 h in well-aerated water, surface sterilized with Rhodiasan (Rhône-Poulenc, Paris, France), and stored at 4°C. On April 15, 1993, the acorns were peeled, soaked in water, and germinated in a peat:sand mixture (1:1, v/v, 5 L) inoculated with mycorrhizal fungi (*Telephora terrestris*). At the same time, a complete fertilization (5 kg m^{-3} slow-release fertilizer, N:P:K, 13:13:13 plus oligoelements; Nutricote 100, Chisso-Asahi Fertilizer Co., Tokyo, Japan) was applied to give optimum nutrition during the experimental period.

The oak seedlings were cultivated for one season in a greenhouse under two plastic tunnels (5 × 3 × 2.3 m) in the presence of ambient or elevated atmospheric CO_2 (350 ± 30 and 700 ± 50 $\mu L\ L^{-1}$). The CO_2 concentration in the tunnels, air temperature, PPFD, and RH were monitored continuously (Picon et al., 1995). The experiment was repeated in 1994. From May 1 to July 20, mean air temperatures were 18.8 ± 1.6 and 19.4 ± 1.7°C in 1993 and 1994, respectively. In this period, global radiation determined outdoors was 1754 ± 70 J $cm^{-2}\ d^{-1}$ (1993) and 1776 ± 73 J $cm^{-2}\ d^{-1}$ (1994). Inside the greenhouse, irradiation was approximately 25% lower. In each year, leaves of the seedlings were collected from the oldest whorl in the 2nd week of July. Sampling took place at approximately noon. In 1993, the plants were subjected to a drought period by withholding water for 6 weeks beginning July 13. Rapid drying of the soil was avoided by covering the top of the pots with waxed paper. The foliage of drought-stressed and watered plants was investigated on August 25, 1993. On each sampling date in 1993, leaves were collected from five individual plants per treatment. In 1994, five independent replicates were investigated per treatment. Each replicate consisted of leaves from four plants. Each of these individual samples was analyzed in triplicate.

One-year-old, container-grown pine (*Pinus pinaster* Ait, provenance Les Landes) seedlings were obtained in 1991 from a nursery near Bordeaux (France), transferred into 5-L pots containing the same soil mixture used for oak, and grown under ambient and elevated CO_2 concentrations as described above. On May 11, 1993, two needle ages (1991 and 1992) were harvested from five individual trees before the emergence of the new flush. In another experiment with pine, 1-year-old pine seedlings were cultivated in the above soil mixture and exposed to ambient and 700 $\mu L\ L^{-1}$ CO_2 from April 1994. The plants were subjected to drought stress from June 16 to July 26 in the presence and absence of elevated CO_2. Ten plants were investigated per treatment. Each of the individual samples was analyzed in triplicate.

Analytical Procedures

Extraction of Enzymes

Fresh oak leaves were ground to a fine powder in liquid nitrogen. Aliquots of 200 mg of frozen leaf powder were transferred into 10 mL of extraction buffer containing 100 mM KH_2PO_4/K_2HPO_4, pH 7.8, 2% Triton X-100, 5 mM ascorbate, and 400 mg of insoluble polyvinylpolypyrrolidone, mixed for 1 min (Vibrofix; Janke and Kunkel, IKA Labortechnik, Staufen, Germany), and incubated on ice for 30 min. The homogenate was centrifuged for 30 min (48,400*g*, 4°C). Pine needles were frozen in liquid nitrogen, transported on dry ice, and stored frozen at −80°C. The needles were extracted in the same manner as the oak leaves but in a buffer containing 1% Triton X-100. The concentration of detergent was varied because the highest enzymatic activities were obtained from oak and pine foliage in the presence of 2 and 1% Triton, respectively. Under these conditions, optimum extraction of soluble protein from oak leaves was also achieved, whereas only 70% of the maximum extractable protein was obtained from pine needles.

Glutathione reductase, ascorbate peroxidase, monodehydroascorbate radical reductase, and dehydroascorbate reductase activities were determined in extracts prepared from fresh leaves. The supernatant was gel filtered over Sephadex G-25 columns (PD-10, Pharmacia) that had been equilibrated with 100 mM KH_2PO_4/K_2HPO_4, pH 7.0 (con-

taining 1 mM ascorbate for the determination of ascorbate peroxidase). Extracts for the determination of catalase, guaiacol peroxidase, and superoxide dismutase activities and the soluble protein content were gel filtered over Sephadex G-25 (PD-10 columns, Pharmacia) with 20 mM KH_2PO_4/K_2HPO_4, pH 7.8, 0.5% Triton and stored at −20°C for analysis. Residual leaf material was frozen in liquid nitrogen and stored at −80°C until analyses of antioxidants and reference parameters.

Determination of Enzymatic Activities

Standard enzymatic assays were performed in a total volume of 1 mL and at 25°C according to the following methods: ascorbate peroxidase (EC 1.11.1.11) (Nakano and Asada, 1987), monodehydroascorbate radical reductase (EC 1.1.5.4) (Borraccino et al., 1989), dehydroascorbate reductase (EC 1.8.5.1) (Dalton et al., 1986), glutathione reductase (EC 1.6.4.2) (Foyer and Halliwell, 1976), catalase (EC 1.11.1.6) (Aebi, 1983), and guaiacol peroxidase (EC 1.11.1.7) (Polle et al., 1990). Blank rates were determined in the absence of either substrate or enzymatic extracts and were subtracted if necessary. The enzymatic activities were calculated with the following extinction coefficients: 2.8, 6.12, 14, 6.12, 39.4, and 25.5 mM for ascorbate peroxidase, monodehydroascorbate radical reductase, dehydroascorbate reductase, glutathione reductase, catalase, and guaiacol peroxidase, respectively. Superoxide dismutase activity (EC 1.15.1.1) was determined by the inhibition of the formation of epinephrine at pH 10.4 and 30°C (Kröniger et al., 1995). One unit of superoxide dismutase activity was defined as the amount of enzyme that inhibited the epinephrine formation by 50%.

Extraction and Determination of Antioxidants

For the extraction of antioxidants, the frozen foliage was ground to a fine powder in liquid nitrogen. The powder (100 mg) was transferred into 5 mL of 0.1 N HCl, 200 mg of polyvinylpolypyrrolidone, 5 μM EDTA, stirred slowly until the mixture was homogeneous, and centrifuged for 45 min (48,400*g*, 4°C). The supernatant was directly used for the determination of reduced ascorbate by HPLC and UV detection at 268 nm (Polle et al., 1992). For the quantification of total ascorbate, additional aliquots of the supernatant were oxidized, derivatized with *o*-phenylenediamine, and determined by fluorescence after HPLC separation (Polle et al., 1990) or alternatively reduced with 60 mM DTT in 200 mM 2-(*N*-cyclohexylamino)ethanesulfonic acid buffer, pH 9.3, for 1 h at room temperature and determined after HPLC separation at 268 nm (Anderson et al., 1992). Total glutathione, γ-glutamylcysteine, and Cys were determined after reduction, derivatization with monobromobimanes, HPLC separation, and fluorescence detection (Schupp and Rennenberg, 1988). GSSG was determined in the same manner after removal of GSH by alkylation with *N*-ethylmaleimide (Gorin et al., 1966). Aliquots of each sample were spiked with known concentrations of ascorbate and thiols. The recoveries of these compounds were 88 ± 17% (ascorbate), 98 ± 11% (ascorbate plus dehydroascorbate), 87 ± 11% (glutathione), 84 ± 14% (Cys), and 95 ± 10% (γ-glutamylcysteine) in oak leaves and 74 ± 9% (ascorbate), 93 ± 7% (ascorbate plus dehydroascorbate), 85 ± 11% (glutathione), 79 ± 17% (Cys), and 97 ± 13% (γ-glutamylcysteine) in pine needles.

Leaf Gas Exchange and Water Potential

CO_2 assimilation rates and conductance for water vapor of oak leaves were measured in situ in the plastic tunnels with a portable photosynthesis chamber (model 6200; Li-Cor, Lincoln, NE) under natural illumination. Only measurements taken at saturating light intensities (>500 μmol quanta m^{-2} s^{-1}) were used to calculate the means shown in tables and figures. The measurements were performed a few days before the collection of the leaves. Gas exchange of pine needles was determined in chambers under controlled conditions. In 1993, measurements were performed in an open system on individual plants using needle age class 1992 as described by Picon et al. (1996). CO_2 was supplied at the same concentrations as under growth conditions. Temperature and PAR were 23°C and 400 μmol quanta m^{-2} s^{-1} (SONT 400-W lamp; Phillips, Eindhoven, The Netherlands). Nine replicates were analyzed per treatment. In 1994, gas exchange of eight pine trees placed in one chamber was recorded together (cf. Vivin et al., 1995) under the following conditions: air temperature 23°C, PAR 500 μmol quanta m^{-2} s^{-1}, and CO_2 concentrations according to growth conditions, i.e. 350 or 700 μL L^{-1}. Predawn water potential was measured on single needles or leaves with a Scholander pressure chamber (Soil Moisture, Santa Barbara, CA).

Basic Parameters

The dry matter of leaves was determined after drying for 72 h at 80°C. The difference between fresh and dry weight was used to calculate the water content of leaves. Pigments were extracted in 80% acetone, and their concentrations were calculated with the extinction coefficients given by Lichtenthaler and Wellburn (1983). Soluble protein was determined with the bicinchoninic acid reagent (Pierce). BSA served as the standard. Leaf area was determined with an area meter (Delta T Device, Cambridge, UK). Statistical analysis was performed with the software STATGRAPHICs (STN, St. Louis, MO) using multiple analysis of variance, followed by an LSD test to evalute significant treatment effects.

RESULTS

The Response of Antioxidative Systems, Soluble Protein, and Pigments in Oak and Pine Foliage to an Elevated Atmospheric CO_2 Concentration

In leaves of oak seedlings raised from acorns under elevated CO_2, the contents of pigments and soluble protein as well as the water content (given in percentage of fresh mass) were similar to those obtained from seedlings grown at an ambient CO_2 concentration (Table I).

Table I. *Photosynthesis, pigments, soluble protein, antioxidants, and activities of antioxidative enzymes in leaves of oak seedlings (Q. robur L.) grown for 5 months at ambient (350 μl L^{-1}) and elevated CO_2 concentrations (700 μl L^{-1}) under greenhouse conditions in two different years*

The leaves were harvested on July 13, 1993 and 1994. Results are means of five individual samples per treatment (±SD) and are related to the dry mass of leaves. ND, Not determined. Photosynthesis was determined in situ under natural light at a PAR of 972 ± 58 μmol quanta $m^{-2}s^{-1}$.

Measurement	Experiment 1993		Experiment 1994	
	Ambient CO_2	700 μl L^{-1} CO_2	Ambient CO_2	700 μl L^{-1} CO_2
Basic parameters				
Photosynthesis (μmol m^{-2} s^{-1})	ND	ND	15.7 ± 3.9[a]	20.9 ± 6.4[b]
Stomatal conductance (μmol m^{-2} s^{-1})	ND	ND	163 ± 51[b]	120 ± 50[a]
Water content (%)	63.8 ± 7.6[a]	63.4 ± 4.6[a]	63.4 ± 3.2[a]	63.1 ± 4.5[a]
Protein (mg g^{-1})	106.6 ± 14.5[a]	93.8 ± 6.3[a]	64.4 ± 31.0[b]	59.1 ± 7.7[b]
Chl (mg g^{-1})	4.15 ± 0.21[a,b]	3.96 ± 0.89[a]	6.07 ± 0.75[b]	5.36 ± 0.96[a,b]
Carotenoids (mg g^{-1})	0.79 ± 0.31[a]	0.81 ± 0.21[a]	1.24 ± 0.15[b]	0.97 ± 0.23[a,b]
Enzymes activities				
Superoxide dismutase (units g^{-1})	3519 ± 611[a]	1844 ± 308[b]	2224 ± 758[b]	604 ± 150[c]
Ascorbate peroxidase (nkat g^{-1})	2032 ± 1181[b]	1225 ± 585[a]	966 ± 376[a]	530 ± 388[a]
Monodehydroascorbate radical reductase (nkat g^{-1})	ND	ND	560 ± 77[a]	518 ± 97[a]
Dehydroascorbate reductase (nkat g^{-1})	ND	ND	not detected	not detected
Glutathione reductase (nkat g^{-1})	88 ± 25[a]	77 ± 34[a]	107 ± 31[a]	92 ± 11[a]
Catalase (μkat g^{-1})	203 ± 46[a]	196 ± 26[a]	169 ± 29[a,b]	120 ± 70[b]
Guaiacol peroxidase (μkat g^{-1})	9.45 ± 1.76[a,b]	10.04 ± 1.51[b]	7.69 ± 2.09[a]	7.70 ± 1.34[a]
Antioxidants				
Total ascorbate (μmol g^{-1})	48.2 ± 13.7[b]	26.0 ± 2.9[a]	29.3 ± 5.1[a]	36.2 ± 4.6[a]
Ascorbate (μmol g^{-1})	16.2 ± 4.0[b]	ND	8.7 ± 4.4[a]	6.6 ± 4.0[a]
Total glutathione (nmol g^{-1})	1970 ± 1115[a]	1325 ± 390[a,b]	1660 ± 740[a,b]	890 ± 240[b]
GSSG (nmol g^{-1})	ND	ND	68.1 ± 46.2[a]	60.6 ± 14.3[a]
Cys (nmol g^{-1})	152.5 ± 83.0[b]	69.5 ± 6.3[a]	72.9 ± 41.6[a]	52.5 ± 16.4[a]
γ-Glutamylcysteine (nmol g^{-1})	56.0 ± 26.5[c]	40.0 ± 15.5[b,c]	20.8 ± 17.1[a,b]	17.6 ± 8.8[a]

[a, b, c] Different letters in rows indicate significant differences at $P \leq 0.05$.

When the same experiment was repeated in a second year, these parameters were also unaffected by the CO_2 concentration, but the absolute content of pigments was significantly higher and the protein content was significantly lower than in the previous year. The magnitude of the enzymatic activities investigated also showed significant differences in both years (Table I). Despite these significant variations in different years, elevated CO_2 always caused significant reductions in the activities of some of the antioxidative enzymes (Table I). For instance, the activities of superoxide dismutases were 2- to 3-fold lower in leaves from oak seedlings grown at 700 μL L^{-1} CO_2 compared to those found in leaves from seedlings cultivated at ambient CO_2 concentrations. The reduction in ascorbate peroxidase activities was less pronounced but nonetheless also significant (Table I). There was also a tendency toward lower activities of other antioxidative enzymes such as monodehydroascorbate radical reductase, glutathione reductase, and catalase in leaves from oak seedling grown at elevated CO_2 concentration, although the effect was not statistically significant on the basis of the five replicates analyzed (Table I).

The total ascorbate content of the leaves (sum of ascorbate plus dehydroascorbate) showed no clear response to CO_2 (Table I). The redox state of the foliar ascorbate pool was low, since 60 to 80% of the total ascorbate in leaves was found to be dehydroascorbate (Table I). Recovery analysis showed that this high degree of oxidation was not caused by unspecific reactions during the extraction procedure, since normal recoveries of reduced ascorbate were observed (see "Materials and Methods"). A low redox state of the ascorbate/dehydroascorbate system is typically found in shade-adapted leaves (A. Polle, unpublished data). Growth at an elevated CO_2 concentration resulted in a significant reduction in the glutathione content of the leaves compared with foliage grown at ambient CO_2 concentrations (Table I). The content of the precursors of glutathione, i.e. Cys and γ-glutamylcysteine, was also diminished. Only 4 to 6% of the total amount of glutathione was present in its oxidized state, suggesting that the plants did not suffer from oxidative stress (Table I).

The effect of elevated CO_2 on basic parameters and key components of the antioxidative system was also analyzed in two needle age classes of maritime pine seedlings (Table II). We observed that the water content and soluble protein content of the leaves were not affected by elevated CO_2. The low protein content in pine needles compared with oak leaves was partially due to incomplete extraction (see "Materials and Methods") and also partially caused by species-specific differences. Maximum protein contents extracted from oak and pine foliage were 40 and 10.5 mg g^{-1} fresh mass, respectively. Other basic parameters such as Chl and carotenoid contents were also lower in pine needles than in oak leaves (cf. Tables I and II). Both 1- and 2-year-old needles from pine seedlings grown at elevated CO_2 con-

Table II. *Photosynthesis, pigments, soluble protein, antioxidants, and activities of antioxidative enzymes in needles of maritime pine seedlings (P. pinaster L.) grown for 24 months at ambient and elevated CO_2 (700 μl L^{-1}) under greenhouse conditions*

The needles were harvested in May 1993. Results are means of five individual plants (±SD) and are related to the dry mass of needles. ND, Not determined. Photosynthesis was determined under controlled conditions at a PAR of 400 μmol quanta m^{-2} s^{-1}.

Measurement	Needle Age Class 1991		Needle Age Class 1992	
	Ambient	700 μl L^{-1}	Ambient	700 μl L^{-1}
Basic parameters				
Photosynthesis (μmol m^{-2} s^{-1})	ND	ND	3.9 ± 0.9	6.2 ± 1.8
Stomatal conductance (mmol m^{-2} s^{-1})	ND	ND	40 ± 14	32 ± 11
Water content (%)	66.5 ± 1.0^a	65.6 ± 2.8^a	70.8 ± 3.2^b	71.4 ± 3.4^b
Protein (mg g^{-1})	8.9 ± 0.7^a	9.3 ± 3.9^a	5.7 ± 0.8^b	4.7 ± 1.4^b
Chl (mg g^{-1})	3.75 ± 0.34^a	3.46 ± 0.98^{a,b}	3.54 ± 0.95^b	2.71 ± 0.41^b
Carotenoids (mg g^{-1})	0.72 ± 0.06^a	0.59 ± 0.05^{a,b}	0.72 ± 0.17^a	0.49 ± 0.18^b
Enzymes activities				
Superoxide dismutase (units g^{-1})	1382 ± 93^b	865 ± 321^a	2632 ± 654^c	1300 ± 193^{a,b}
Catalase (μkat g^{-1})	32.5 ± 4.4^a	28.7 ± 7.0^a	28.7 ± 6.2^a	26.2 ± 3.7^a
Guaiacol peroxidase (μkat g^{-1})	15.67 ± 2.47^a	10.9 ± 2.49^b	0.17 ± 0.16^c	0.06 ± 0.02^c
Antioxidants				
Total ascorbate (μmol g^{-1})	53.8 ± 9.6^b	46.7 ± 10.3^{a,b}	40.1 ± 6.2^a	38.6 ± 7.2^a
Ascorbate (μmol g^{-1})	49.6 ± 6.7^c	44.5 ± 9.2^{b,c}	36.1 ± 4.7^{a,b}	33.0 ± 6.0^a
Total glutathione (nmol g^{-1})	804 ± 341^a	1059 ± 76^{a,b}	1070 ± 749^{a,b}	1525 ± 570^b
GSSG (nmol g^{-1})	16 ± 7^a	27 ± 11^a	33 ± 23^a	24 ± 13^a
Cys (nmol g^{-1})	109 ± 45^b	138 ± 89^b	52 ± 28^a	67 ± 22^a
γ-Glutamylcysteine (nmol g^{-1})	59 ± 33^a	31 ± 13^{a,b}	15 ± 3^b	17 ± 26^b

$^{a, b, c}$ Different letters in rows indicate significant differences at $P \leq 0.05$.

centrations contained significantly less Chl and carotenoids than did needles from plants grown at ambient CO_2, but photosynthesis was still higher in shoots of seedlings grown at elevated CO_2 compared with those grown at ambient CO_2 concentrations (Table II).

In both needle age classes, the activity of superoxide dismutase was decreased in response to elevated CO_2. However, the effect was more pronounced in 1- than in 2-year-old pine needles (Table II). The activities of catalase and "nonspecific" peroxidases and the content of ascorbate also tended to decrease, although this effect was not always significant (Table II). Unlike in oak leaves, in needles of pine seedlings grown at elevated CO_2 concentrations the content of glutathione increased (Table II).

The Response of Photosynthesis, Pigments, and Antioxidants to Drought Stress in Combination with Elevated CO_2

In leaves of the young oak trees, the effect of water shortage was analyzed after a drought period of about 6 weeks. Severe drought stress had not developed, since the predawn water potentials were still similar to those of the watered plants (Table III). In contrast, pine needles already suffered from severe drought stress with water potentials of less than −2 MPa after 4 weeks of water shortage (Table IV). The faster development of drought stress in pine was presumably related to an approximately 1.7-fold greater leaf surface compared to that of oak (0.14 and 0.20 m^2 pine $plant^{-1}$ grown at ambient and elevated CO_2, respectively [P. Vivin, unpublished results] and 0.08 and 0.11 m^2 oak $plant^{-1}$ grown at ambient and elevated CO_2, respectively [Picon et al., 1995]). Water content and soluble protein content of leaves from drought-treated plants were similar to those of well-watered plants (Tables III and IV). In oak, the Chl and carotenoid content per gram of dry mass was increased in stressed leaves as compared with leaves from watered plants (Table III). A similar trend was observed in drought-treated pine needles. However, the difference between stressed and unstressed leaves disappeared if the pigment content was related to the leaf area (Table III).

Table III. *Basic parameters in leaves of pendunculate oak (Q. robur L.) grown for 5 months with ambient and elevated CO_2 (700 μl L^{-1}) in a greenhouse and subjected to drought stress from July 13 to August 26, 1993*

Results are means of five individual plants (±SD) and are related to the dry mass of leaves. CO_2 concentrations: A, ambient; E, elevated (700 μl L^{-1}). Water supply: W, watered regularly; D, not watered.

Parameter	A-W	A-D	E-W	E-D
Water content (%)	58.6 ± 1.8^a	61.0 ± 3.7^a	61.6 ± 5.4^a	59.6 ± 2.4^a
Predawn water potential (MPa)	−0.64 ± 0.15^a	−0.79 ± 0.24^{a,b}	−0.69 ± 0.20^a	−0.91 ± 0.26^b
Leaf mass area ratio (g m^{-2})	84.0 ± 8.7^b	74.7 ± 5.3^a	74.3 ± 12.8^a	68.5 ± 5.7^a
Chl (mg g^{-1})	3.39 ± 0.32^a	3.45 ± 0.96^a	4.17 ± 0.84^{a,b}	4.96 ± 0.93^a
Carotenoids (mg g^{-1})	0.75 ± 0.09^a	0.81 ± 0.21^a	0.87 ± 0.15^{a,b}	0.93 ± 0.19^b
Soluble protein (mg g^{-1})	101.5 ± 15.9^a	89.9 ± 23.1^a	93.3 ± 21.5^a	99.5 ± 20.2^a

$^{a, b}$ Different letters in rows indicate significant differences at $P \leq 0.05$.

Table IV. *Basic parameters determined in needle age class 1994 of maritime pine seedlings (P. pinaster L.) grown for 6 months with ambient and elevated CO_2 (700 μl L^{-1}) under greenhouse conditions and subjected to drought stress from June 16 to July 26, 1994*

Results are means of 10 individual plants (±SD) and are expressed on the basis of dry matter. CO_2 concentrations: A, ambient; E, elevated (700 μl L^{-1}). Water supply: W, watered regularly; D, not watered.

Parameter	A-W	A-D	E-W	E-D
Water content (%)	69.9 ± 4.5[b]	69.7 ± 3.4[b]	66.7 ± 5.0[a,b]	63.6 ± 6.6[a]
Predawn water potential (MPa)	−0.56 ± 0.41[a]	−2.59 ± 0.83[b]	−0.83 ± 0.23[a]	−2.40 ± 0.23[b]
Leaf mass area ratio (g m^{-2})	236 ± 10[a]	226 ± 10[a]	215 ± 10[a]	252 ± 8[a]
Chl (mg g^{-1})	1.84 ± 0.22[a]	1.99 ± 0.54[a]	1.57 ± 0.67[a]	1.92 ± 0.25[a]
Carotenoids (mg g^{-1})	0.48 ± 0.02[a]	0.46 ± 0.07[a,b]	0.39 ± 0.07[a]	0.39 ± 0.10[a]
Soluble protein (mg g^{-1})	10.0 ± 2.4[c]	8.34 ± 1.64[b,c]	6.06 ± 3.14[a]	6.45 ± 1.81[a,b]
Weight (g $plant^{-1}$)	25.7 ± 2.9[a]	27.4 ± 3.1[a]	36.4 ± 6.2[b]	38.7 ± 2.8[b]

[a, b, c] Different letters in rows indicate significant differences at $P \leq 0.05$.

In oak, the effect of withholding water was apparent from reduced stomatal conductivities for water vapor (Table III) and a significant reduction in the actual rate of net CO_2 assimilation in stressed compared with unstressed leaves (Fig. 1A). In unstressed leaves measured in August the rate of photosynthesis was similar at both CO_2 concentrations (Fig. 1A), whereas earlier in the season a significant enhancement of photosynthesis in leaves of oak seedlings grown at 700 μL L^{-1} CO_2 was observed (Table I, experiment 1994).

Leaves from young oak trees grown at ambient CO_2 and subjected to drought stress contained significantly decreased superoxide dismutase and catalase activities and a decreased total content of ascorbate, whereas the amount of reduced ascorbate increased compared with well-watered plants (Fig. 1, B and C). Other components of the antioxidative system such as ascorbate peroxidase, glutathione reductase, glutathione, and guaiacol peroxidase were not affected by mild drought stress (Fig. 1, D–G). In leaves from oak seedlings grown at an elevated CO_2 concentration, in which the activities of superoxide dismutase and ascorbate peroxidase were low in unstressed plants, significant enhancements of these enzymatic activities were observed in response to drought (Fig. 1, B and G). The content of reduced ascorbate increased as well (Fig. 1H). Catalase, guaiacol peroxidase, and glutathione reductase activities and the total content of glutathione were not affected (Fig. 1, C–F). The content of GSSG was not determined in this experiment, but in pine needles, which were subjected to more pronounced drought stress than oak leaves, the content of GSSG was not significantly affected in needles from drought-treated plants (Fig. 2F).

Pine seedlings grown at elevated CO_2 concentrations showed a stimulation of photosynthesis (Fig. 2A). Because of stomatal closure (cf. Table IV), net CO_2 assimilation was not observed in severely drought-stressed pine seedlings (Fig. 2A). In needles of pine seedlings grown at ambient CO_2 concentration and subjected to drought stress, the activity of superoxide dismutase was decreased compared to that of needles from well-watered trees (Fig. 2B). By contrast, superoxide dismutase activity in needles of pine grown at an elevated CO_2 concentration increased in needles of trees additionally subjected to drought stress (Fig. 2B). Drought stress caused significant decreases in catalase activity both in pine trees grown with ambient CO_2 concentrations and in those grown at elevated CO_2 concentrations.

In contrast to the situation in oak, the activities of guaiacol peroxidases showed significant changes in pine needles in response to drought stress in combination with elevated CO_2 concentration. The content of total glutathione increased in response to drought stress in needles of plants grown at ambient CO_2 (Fig. 2F). In pine needles of trees cultivated with elevated CO_2 concentration, the glutathione content was not affected by drought stress and the size of the glutathione pool was similar in plants cultivated with ambient and elevated CO_2 concentrations.

DISCUSSION

Responses to Elevated CO_2 in Unstressed Leaves

In the present study, the effect of elevated atmospheric CO_2 on photosynthesis and antioxidative systems was addressed in oak and pine seedlings as representatives of deciduous and evergreen tree species. Replications of the experiments showed that basic parameters such as Chl, carotenoids, and soluble protein contents as well as activities of antioxidative enzymes in plants grown at ambient CO_2 concentrations showed considerable differences in different years and in samples collected at different dates in the same year (cf. Table I for oak and Table II and Fig. 2 for pine). Reasons for the observed variations might have been differences in ambient climatic conditions in the greenhouse or differences in the developmental stage of leaves. Leaf age has a profound effect on the antioxidative capacity (Polle and Morawe, 1995; Polle, 1995). Oak leaves collected in July 1993 contained higher antioxidant levels than leaves collected in August 1993 (cf. Table I and Fig. 2). An age-dependent decrease in the activity of superoxide dismutase was observed in pine needles (Table II) and is similar to that found in spruce (Polle et al., 1989). Relatively large fluctuations in the activities of antioxidative enzymes have been reported in mature leaves from field-grown beech trees during several seasons (Polle and Morawe, 1995) and in bean leaves measured at different times of the day (Badiani et al., 1993).

In the presence of elevated CO_2, pine seedlings showed a stimulation of photosynthesis, which persisted even after

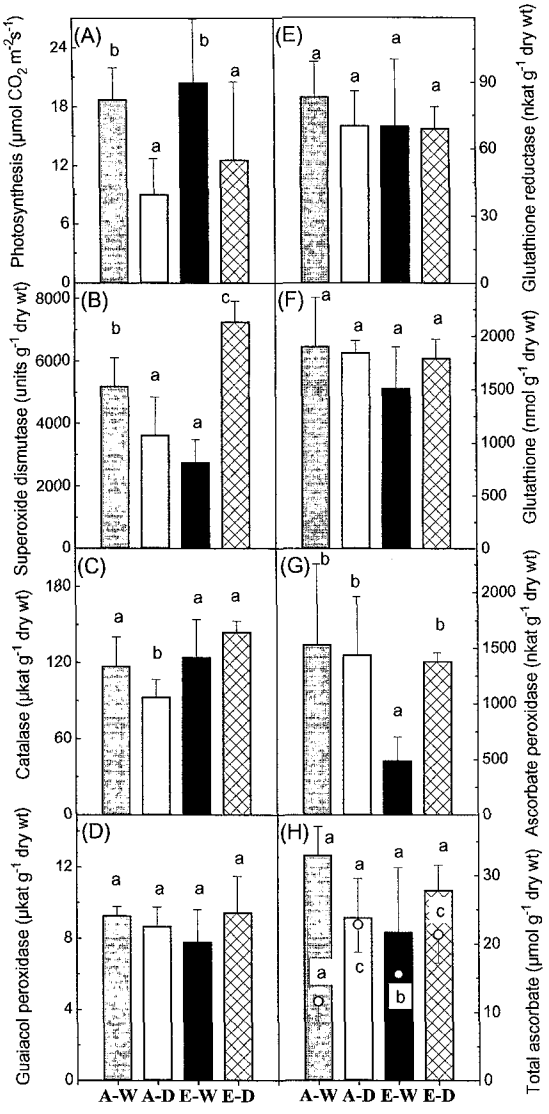

Figure 1. Interactive effects of drought stress and elevated CO_2 on photosynthesis and on antioxidative enzymes and their substrates in leaves of oak seedlings (*Q. robur*, L.). Photosynthesis was measured in situ under natural illumination of 1125 ± 56 μmol m^{-2} s^{-1}. Bars indicate means (±SD, n = 5). Circles in H refer to reduced ascorbate. Other conditions are as in Table III. Different lowercase letters indicate significant differences at $P \leq 0.05$. CO_2 concentrations: A, ambient; E, elevated (700 μL L^{-1}). Water supply: W, watered regularly; D, not watered.

2 years (Table II; cf. Guehl et al., 1994). Initially, a stimulation of photosynthesis due to CO_2 enhancement was also observed in oak (Table I), but at a later sampling date this increase was no longer apparent (Fig. 2A). An "acclimation" of photosynthesis has frequently been observed under the influence of elevated CO_2 concentrations and was generally associated with a loss in Rubisco protein (Webber et al., 1994). However, in the present study detailed measurements of photosynthesis of oak leaves showed considerable fluctuations of the increment in photosynthesis in plants grown at 700 μL L^{-1} CO_2 compared to plants grown at ambient CO_2 during the course of the experiment (Picon et al., 1995).

Despite these fluctuations, the present results clearly show that elevated CO_2 caused significant reductions in superoxide dismutase activity regardless of whether pine needles or oak leaves were analyzed, regardless of leaf age, and regardless of whether photosynthesis was enhanced. The responses of the other components of the antioxidative system to elevated CO_2 were less clear. Increases in antioxidants in plants grown at 700 μL L^{-1} CO_2 were observed only once (i.e. an increase in glutathione in pine needles; Table II) and once an increase in the redox state of the ascorbate pool in oak leaves was observed (Fig. 2). In most cases analyzed, ascorbate and glutathione and the enzymes responsible for the reduction of these antioxidants were not affected by elevated CO_2, whereas catalase and ascorbate peroxidase activities were decreased. Apparently, elevated CO_2 resulted in a reduction in antioxidative defenses rather than in increases as one might have assumed because of the better supply of plants with photosynthetate.

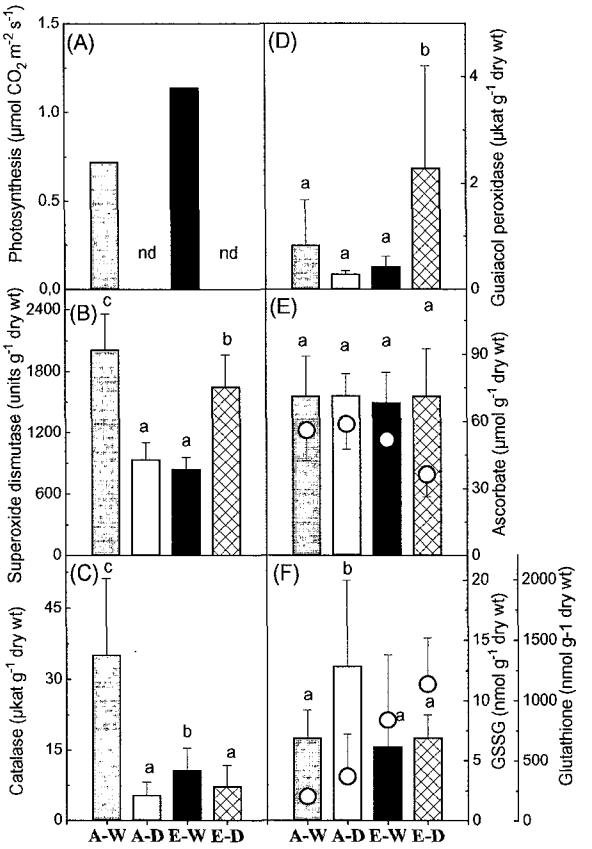

Figure 2. Interactive effects of drought stress and elevated CO_2 on photosynthesis and on antioxidative enzymes and their substrates in leaves of pine seedlings (*P. pinaster*, L.). Photosynthesis (A) was measured at a PAR of 500 μmol quanta m^{-2} s^{-1} under controlled conditions in a chamber on eight trees together. Bars in B to F indicate means (±SD, n = 10). Circles in E and H refer to reduced ascorbate and GSSG, respectively. Other conditions are as in Table III. Different lowercase letters indicate significant differences at $P \leq 0.05$. nd, Not detected; CO_2 concentrations: A, ambient; E, elevated (700 μL L^{-1}). Water supply: W, watered regularly; D, not watered.

Reductions in superoxide dismutase and catalase activities have also been observed in spruce needles grown at 750 μL L^{-1} CO_2 (Polle et al., 1993). Some recent data suggest that the reduction in antioxidative enzymes might depend on the degree of CO_2 enhancement and the nutritional stage of the plants (Schwanz et al., 1996). A reduction in catalase but not in superoxide dismutase activity has been found in tobacco leaves exposed for 2 d to an elevated CO_2 concentration (Havir and McHale, 1989). It has been suggested that less catalase activity might be required for H_2O_2 detoxification because of suppression of photorespiration when Rubisco activity is shifted toward carboxylation under an elevated CO_2 concentration (Polle et al., 1993). Furthermore, one might argue that an enhanced use of reductant for carboxylation might enhance the availability of $NADP^+$ as electron acceptor at PSI and, thereby, limit the flux of electron to molecular O_2. If the Mehler reaction were suppressed, less detoxification of $O_2^{\cdot-}$ via superoxide dismutase and the ascorbate-glutathione-system might be required to maintain the concentration of $O_2^{\cdot-}$ at a low level. This hypothesis is tempting because it would explain the observed reduction in ascorbate peroxidase and superoxide dismutase activities (Tables I and II; Figs. 2 and 3). Further studies will be required to determine which factors regulate the flux of electrons to molecular O_2, but the present results suggest a link to carboxylation efficiency. Based on the assumption that the concentration of $O_2^{\cdot-}$ in healthy tissues is maintained at a low but constant level, the present results strongly suggest that plants grown with elevated CO_2 suffer less oxidative stress than plants grown at ambient atmospheric CO_2 concentrations.

The molecular mechanisms causing down-regulation of superoxide dismutase activity at an elevated CO_2 concentration are currently unknown. Reduced glutathione and other sulfhydryl reagents have been shown to serve as signals for the regulation of superoxide dismutase activity (Hérouart et al., 1993). However, the present results show no connection between the glutathione content or the redox state of the glutathione pool and the activity of superoxide dismutase, since the glutathione content was enhanced in pine and reduced in oak leaves but both species showed significant reductions in superoxide dismutase activity (Tables I and II).

Responses to Drought Stress at Elevated CO_2

Plants with low antioxidative capacity were found to confer lower stress tolerance than plants with high antioxidative capacity (Shaaltiel et al., 1988). For example, transgenic tobacco plants with decreased activity of glutathione reductase were more susceptible to paraquat-induced oxidative stress than the wild type (Aono et al., 1995). Therefore, a reduced antioxidative capacity in plants grown with elevated CO_2 concentrations may be alarming with respect to future climatic scenarios. To test whether plants grown at elevated CO_2 concentrations with low superoxide dismutase activity might be more susceptible to oxidative stress than plants grown at ambient CO_2 concentrations, oak and pine seedlings were subjected to drought stress. Drought stress can result in an increased production of reactive O_2 species and, therefore, appears to require elevated levels of antioxidants for stress compensation (Smirnoff, 1993). A drought-tolerant maize strain responded with significant increases in antioxidants to water deficits, whereas a susceptible strain maintained a lower protection from oxidants (Pastori and Trippi, 1992). In drying acorns of *Q. robur*, a loss in viability was found to be associated with a significant reduction in antioxidative defenses and the appearance of free radicals (Hendry et al., 1992). The present results show that drought caused reductions in both superoxide dismutase and catalase activities in oak leaves and pine needles grown at ambient CO_2 (Figs. 2 and 3). This response was unexpected because oak is known as an extremely drought-resistant species whose leaves maintain photosynthetic capacity even during extended periods of water deficits leading to predawn water potentials of less than −2.0 MPa (Epron and Dreyer, 1993b). In the present study a mild drought stress was applied that did not affect the predawn water potential of oak (Table III) and, thus, was unlikely to have caused tissue injury. Although pine needles suffered from greater drought stress (Table IV) than oak leaves, there was also no evidence for tissue injury because pigment and protein contents were not affected (Table IV), lipid peroxidation was not increased (P. Schwanz, unpublished data), and the redox state of antioxidants remained unchanged compared with well-watered plants (Fig. 2). The observed reduction in antioxidants is clearly at variance with results obtained with herbaceous plants. For instance, Mittler and Zilinskas (1994) showed an increased production of mRNA for ascorbate peroxidase and superoxide dismutase and enhanced enzymatic activity of these proteins in drought-stressed pea plants. Catalase activity was also increased (Mittler and Zilinskas, 1994). These observations support the idea that both the Mehler reaction and photorespiration are important metabolic pathways for dissipation of light energy when the flux of CO_2 into the leaves of herbaceous plants is limited under drought conditions (Foyer and Harbinson, 1994). Apparently, leaves of tree species respond to drought in a different way. One reason might be that responses to water deficits develop slowly over 4 to 6 weeks in the sclerophyllous foliage of pine and oak but within only a few days in tender leaves of peas; therefore, in one case plants might gradually adjust to changing conditions, whereas in the other case the change is relatively abrupt and might exceed the metabolic capacity of plants to acclimate.

Recently, it has been shown by combined measurements of fluorescence and CO_2 assimilation that drought resulted in a decrease in total photosynthetic electron flux and photorespiration in turkey oak under field conditions (Valentini et al., 1995). The ratio of oxygenase to carboxylase activity of Rubisco was shifted toward oxygenation (Valentini et al., 1995). It is conceivable that the reductions in superoxide dismutase and catalase activities observed in the present study (Figs. 2 and 3) reflect an acclimation to such a down-regulation of total electron flux under drought conditions. Our results together with those of Valentini et al. (1995) suggest that the overall necessity for scavenging of reactive O_2 species from the Mehler reaction

or photorespiration might be diminished in drought-stressed oak leaves and that the relative importance of down-regulation of PSII efficiency, photorespiration, or other protective mechanisms for energy dissipation is greater than that of the Mehler reaction. Given the decreased protection from $O_2^{\cdot -}$ and H_2O_2, it is apparent that increasing drought, which may eventually cause a release of Fenton metals such Fe or Cu and result in oxidative stress and membrane deterioration (Price and Hendry, 1991; Moran et al., 1994), puts leaves of tree species at a severe risk for unspecific oxidation.

In contrast to drought applied under ambient CO_2, drought stress applied under an elevated CO_2 concentration resulted not in decreases but in increases in superoxide dismutase and ascorbate peroxidase activities up to the levels observed in unstressed plants grown at ambient CO_2 concentrations (Figs. 2 and 3). It is possible that intrinsic, developmentally determined factors set a ceiling to the magnitude of the response of the antioxidative systems, since antioxidant levels found in unstressed leaves under ambient conditions were not exceeded. Based on the assumption that superoxide dismutase activity reflects the need for detoxification of $O_2^{\cdot -}$, one must conclude that the rate of $O_2^{\cdot -}$ production was increased in plants grown with elevated CO_2 and subjected to drought stress. Perhaps plants grown at an elevated CO_2 concentration contain increased metabolic reserves and, thus, might have an enhanced metabolic flexibility to respond to oxidative stress compared with plants grown at ambient CO_2 concentrations. If elevated CO_2 renders plants more flexible to cope with environmental stresses such as drought, this will have important economic and ecological benefits. On the other hand, it will be important to study whether the responsiveness of the protective systems in plants grown at elevated CO_2 concentrations is also sufficient to cope with oxidative stresses that fluctuate more rapidly, such as ozone.

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LITERATURE CITED

Aebi H (1983) Catalase. *In* H Bergmeyer, ed, Methods of Enzymatic Analysis, Vol 3. Verlag Chemie, Weinheim, Germany, pp 273–277

Alscher RG, Madamachani NR, Cramer CL (1991) Protective mechanisms in the chloroplast stroma. *In* E Pell, K Steffen, eds, Current Topics in Plant Physiology, Vol 6: Active Oxygen/Oxidative Stress and Plant Metabolism. American Society of Plant Physiologists, Rockville, MD, pp 145–155

Anderson JV, Chevone BI, Hess JL (1992) Seasonal variation in the antioxidant system of eastern white pine. Plant Physiol **98:** 501–508

Aono M, Saji H, Fuijama K, Sugita M, Kondo N, Tanaka K (1995) Decrease in activity of glutathione reductase enhances paraquat sensitivity in transgenic *Nicotiana tabacum*. Plant Physiol **197:** 645–648

Badiani M, Schenone G, Paolacci R, Fumagalli I (1993) Daily fluctuations of antioxidants in bean (*Phaseolus vulgaris* L.) leaves as affected by the presence of ambient air pollutants. Plant Cell Physiol **34:** 271–279

Borraccino G, Dipierro S, Arrigoni O (1989) Interaction of ascorbate free radical reductase with sulphhydryl reagents. Phytochemistry **28:** 715–717

Chaves M, Pereira J (1992) Water stress, CO_2, and climate change. J Exp Bot **43:** 1131–1139

Cornic G, Le Gouallec JL, Briantais JM, Hodges M (1989) Effect of dehydration and high light on photosynthesis of two C_3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) hall f.). Planta **177:** 84–90

Dalton D, Russell S, Hanus F, Pascoe G, Evans H (1986) Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soy bean root nodules. Proc Natl Acad Sci USA **83:** 3811–3815

Elstner EF, Osswald W (1994) Mechanisms of oxygen activation during plant stress. Proc R Soc Edinb Sect B **102:** 131–154

Epron D, Dreyer E (1993a) Long-term effects of drought on photosynthesis of adult oak trees [*Quercus petrea* (Matt.) Liebl. and *Quercus robur* L.] in a natural stand. New Phytol **125:** 381–389

Epron D, Dreyer E (1993b) Photosynthesis of oak leaves under water stress: maintenance of high photochemical efficiency of photosystem II and occurrence of stomatal patchiness. Tree Physiol **13:** 107–119

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta **133:** 21–25

Foyer CH, Harbinson J (1994) Oxygen metabolism and the regulation of photosynthetic electron transport. *In* C Foyer, P Mullineaux, eds, Causes of Photo-oxidative Stress and Amelioration of Defense Systems in Plants. CRC Press, Boca Raton, FL, pp 1–43

Gorin G, Martic PA, Doughty G (1966) Kinetics of the reaction of *N*-ethylmaleimide with cysteine and some congeners. Arch Biochem Biophys **115:** 593–597

Guehl JM, Picon C, Aussenac G, Gross P (1994) Interactive effects of elevated CO_2 and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. Tree Physiol **14:** 707–724

Havir EA, McHale NA (1989) Regulation of catalase activity in leaves of *Nicotiana sylvestris* by high CO_2. Plant Physiol **89:** 952–957

Hendry GA, Finch-Savage WE, Thorpe C, Atherton NM, Buckland S, Nilsson KA, Seel WE (1992) Free radical processes and loss of seed viability during desiccation in the recalcitrant species *Quercus robur* L. New Phytol **122:** 273–279

Hérouart D, van Montagu M, Inzé D (1993) Redox-activated expression of the cytosolic copper/zinc superoxide dismutase gene in *Nicotiana*. Proc Natl Acad Sci USA **90:** 3108–3112

Kaiser WM (1987) Effect of water deficit on photosynthetic capacity. Physiol Plant **71:** 142–149

Kröniger W, Rennenberg H, Tadros H, Polle A (1995) Purification and properties of manganese superoxide dismutase from spruce (*Picea abies*, L. Karst.). Plant Cell Physiol **36:** 191–196

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. Biochem Soc Trans **603:** 591–592

Mittler R, Zilinskas B (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant J **5:** 397–405

Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas R, Aparicio-Tejo P (1994) Drought induces oxidative stress in pea plants. Planta **194:** 346–352

Nakano Y, Asada K (1987) Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiol **28:** 131–140

Pastori G, Trippi V (1992) Oxidative stress induced high rate of glutathione reductase synthesis in a drought-resistant maize strain. Plant Cell Physiol **33:** 957–961

Pfündel E, Bilger W (1994) Regulation and possible function of the violaxanthin cycle. Photosynth Res **42:** 89–109

Picon C, Guehl JM, Aussenac G (1996) Growth dynamics, transpiration and water-use efficiency in *Quercus robur* plants submitted to elevated CO_2 and drought. Ann Sci For (in press)

Picon C, Guehl JM, Ferhi A (1996) Leaf gas exchange and carbon isotope composition responses to drought in a drought-avoiding (*Pinus pinaster*) and a drought-tolerant (*Quercus petrea*) species under present and elevated atmospheric CO_2 concentrations. Plant Cell Environ (in press)

Polle A (1995) Developmental changes of the antioxidative system in tobacco leaves as affected by limited sucrose export in transgenic tobacco expressing yeast-invertase in the apoplastic space. Planta **197:** (in press)

Polle A, Chakrabarti K, Chakrabarti S, Seifert F, Schramel P, Rennenberg H (1992) Antioxidants and manganese deficiency in needles of Norway spruce (*Picea abies*, L.) trees. Plant Physiol **99:** 1084–1089

Polle A, Chakrabarti K, Schürmann W, Rennenberg H (1990) Composition and properties of hydrogen peroxide decomposing systems in extracellular and total extracts from needles of Norway spruce (*Picea abies*, L. Karst.). Plant Physiol **94:** 312–319

Polle A, Krings B, Rennenberg H (1989) Superoxide dismutase activity in needles of Norwegian spruce trees (*Picea abies* L.). Plant Physiol **90:** 1310–1315

Polle A, Morawe B (1995) Seasonal changes of antioxidative systems in foliar buds and leaves of beech trees (*Fagus sylvatica*, L.) in a stressful climate. Bot Acta **108:** 314–320

Polle A, Pfirrmann T, Chakrabarti S, Rennenberg H (1993) The effects of enhanced ozone and enhanced carbon dioxide concentrations on biomass, pigments and antioxidative enzymes in spruce needles (*Picea abies*, L.). Plant Cell Environ **16:** 311–316

Price A, Hendry GA (1991) Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. Plant Cell Environ **14:** 477–484

Quartacci M, Navari-Izzo F (1992) Water stress and free radical mediated changes in sun flower seedlings. J Plant Physiol **139:** 621–625

Quick WP, Chaves M, Wendler R, David M, Rodrigues ML, Passarinho JA, Pereira JS, Adcock MD, Leegood RC, Stitt M (1992) The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. Plant Cell Environ **15:** 25–35

Rochefort L, Woodward FI (1992) Effects of climate change and a doubling of CO_2 on vegetation diversity. J Exp Bot **43:** 1169–1180

Roeckner E (1992) Past, present and future levels of greenhouse gases in the atmosphere and model projections of related climatic changes. J Exp Bot **43:** 1097–1109

Schupp R, Rennenberg H (1988) Diurnal changes in the glutathione content of spruce needles (*Picea abies*, L.). Plant Sci **57:** 113–117

Schwanz P, Häberle KH, Polle A (1996) Interactive effects of elevated CO_2, ozone and drought stress on the activities of antioxidative enzymes in needles of Norway spruce trees (*Picea abies* [L.] Karsten) grown with luxurious N-supply. J Plant Physiol (in press)

Seegmüller S, Rennenberg H (1994) Interactive effects of mycorrhization and elevated carbon dioxide on growth of young pendunculate oak (*Quercus robur* L.) trees. Plant Soil **167:** 325–329

Shaaltiel Y, Glazer A, Bozion P, Gressel J (1988) Cross-tolerance to herbicidal and environmental oxidants of plant biotypes tolerant to paraquat, sulfur dioxide and ozone. Pestic Biochem Physiol **31:** 13–33

Smirnoff N (1993) The role of active oxygen in response of plants to water deficit and desiccation. New Phytol **125:** 27–58

Valentini R, Epron D, De Angelis P, Matteucci G, Dreyer E (1995) In situ estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in turkey oak (*Q. cerris* L.) leaves: diurnal cycles under different levels of water supply. Plant Cell Environ **18:** 631–640

Vivin P, Gross P, Aussenac G, Guehl JM (1995) Whole plant CO_2 exchange, carbon partitioning and growth in *Quercus robur* seedlings exposed to elevated CO_2. Plant Physiol Biochem **33:** 201–211

Webber AN, Nie G-Y, Long SP (1994) Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynth Res **39:** 413–425