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Acclimation of photosynthesis in relation to Rubisco and non-structural carbohydrate contents and *in situ* carboxylase activity in *Scirpus olneyi* grown at elevated CO_2 in the field

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ABSTRACT

Stands of *Scirpus olneyi*, a native saltmarsh sedge with C_3 photosynthesis, had been exposed to normal ambient and elevated atmospheric CO_2 concentrations (C_a) in their native habitat since 1987. The objective of this investigation was to characterize the acclimation of photosynthesis of *Scirpus olneyi* stems, the photosynthesizing organs of this species, to long-term elevated C_a treatment in relation to the concentrations of Rubisco and non-structural carbohydrates. Measurements were made on intact stems in the field under existing natural conditions and in the laboratory under controlled conditions on stems excised in the field early in the morning. Plants grown at elevated C_a had a significantly higher (30–59%) net CO_2 assimilation rate (A) than those grown at ambient C_a when measurements were performed on excised stems at the respective growth C_a. However, when measurements were made at normal ambient C_a, A was smaller (45–53%) in plants grown at elevated C_a than in those grown at ambient C_a. The reductions in A at normal ambient C_a, carboxylation efficiency and *in situ* carboxylase activity were caused by a decreased Rubisco concentration (30–58%) in plants grown at elevated C_a; these plants also contained less soluble protein (39–52%). The Rubisco content was 43 to 58% of soluble protein, and this relationship was not significantly altered by the growth CO_2 concentrations. The Rubisco activation state increased slightly, but the *in situ* carboxylase activity decreased substantially in plants grown at elevated C_a. When measurements were made on intact stems in the field, the elevated C_a treatment caused a greater stimulation of A (100%) and a smaller reduction in carboxylation efficiency (which was not statistically significant) than when measurements were made on excised stems in the laboratory. The possible reasons for this are discussed.

Plants grown at elevated C_a contained more non-structural carbohydrates (25–53%) than those grown at ambient C_a. Plants grown at elevated C_a appear to have sufficient sink capacity to utilize the additional carbohydrates formed during photosynthesis.

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Overall, our results are in agreement with the hypothesis that elevated C_a leads to an increased carbohydrate concentration and the ensuing acclimation of the photosynthetic apparatus in C_3 plants results in a reduction in the protein complement, especially Rubisco, which reduces the photosynthetic capacity in plants grown at elevated C_a, relative to plants grown at normal ambient C_a. Nevertheless, when compared at their respective growth C_a, *Scirpus olneyi* plants grown at elevated C_a in their native habitat maintained a substantially higher rate of photosynthesis than those grown at normal ambient C_a even after 8 years of growth at elevated C_a.

Key-words: Scirpus olneyi; acclimation; carboxylation efficiency; elevated CO_2; photosynthesis; Rubisco, soluble proteins; soluble sugars; starch.

INTRODUCTION

The present CO_2 concentration in the atmosphere (C_a) is generally limiting to C_3 photosynthesis, primarily because of the well-known bi-functional nature of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) and its low affinity for CO_2 (Woodrow & Berry 1988; Bowes 1993). An increase in C_a increases the leaf internal CO_2 concentration (C_i) and the $[CO_2]/[O_2]$ ratio at the Rubisco site which favours carboxylation rather than oxygenation of RuBP catalysed by Rubisco (Andrews & Lorimer 1987). Assuming no other limitations, an increase in C_a will increase photosynthesis through its direct effect on Rubisco (Farquhar, von Caemmerer & Berry 1980; Woodrow & Berry 1988; Bowes 1993).

While short-term growth at elevated C_a increases the rate of photosynthetic CO_2 assimilation per unit leaf area (A) in C_3 plants, some studies show that this increase may not be maintained in the long term as a result of acclimation in the photosynthetic apparatus (Kramer 1981; DeLucia, Sasek & Strain 1985; Sage, Sharkey & Seemann 1989; Yelle *et al.* 1989a). A decrease in the amounts of Rubisco and tissue N and an increase in the amount of carbohydrates are observed, often associated with depression of photosynthesis in leaves acclimated to elevated C_a by long-term exposure (Wong 1979; Spencer & Bowes 1986;

Yelle *et al.* 1989a,b; Besford, Ludwig & Withers 1990; Rowland-Bamford *et al.* 1991; Tissue, Thomas & Strain 1993). However, a recent review of the data on photosynthesis (Long & Drake 1992) showed that, in general, C_3 plants grown and measured at elevated C_a maintained a substantial stimulation of A (with an average increase of 51%) compared with plants grown and measured at ambient C_a, but with considerable variation in the response of photosynthesis and Rubisco to elevated C_a. It is unclear how these variations relate to species, developmental stage of the plant, environmental conditions, resource availability, or internal factors such as source–sink balance (Bowes 1991; Long 1991; Stitt 1991).

Stitt (1991) proposed a conceptual model to describe the long-term acclimation of photosynthesis to elevated C_a in relation to changes in the source–sink balance in the whole plant. According to this model, changes in source–sink balance can lead to accumulation of large amounts of carbohydrates in the leaves which can inhibit photosynthesis by feed-back regulation and may also affect the level of expression of various photosynthesis genes, including those of Rubisco (Stitt & Schulze 1994). For example, addition of glucose to autotrophic cell suspension cultures of *Chenopodium* suppressed the transcription of the gene coding for the small subunit of Rubisco (Krapp *et al.* 1993).

While a large number of studies on the effects of growing plants in elevated C_a have been conducted using crop plants, few studies have addressed photosynthetic acclimation to long-term elevated C_a treatment in native species growing in their natural habitat. The present investigation was conducted using *Scirpus olneyi*, a salt marsh sedge with the C_3 photosynthetic pathway, which has been continuously exposed throughout its growing season (May to October) to elevated C_a in its native habitat beginning in 1987. Our major objective in this study was to determine how long-term elevated C_a treatment in the field affects photosynthetic constituents (e.g. Rubisco and carbohydrates) and whether changes in their contents would result in fundamentally different rates of photosynthesis in plants grown in elevated and in ambient C_a. We measured the rate of water vapour and CO_2 exchange by *Scirpus* stems, the photosynthesizing organs of this species, and expressed A as a function of C_i. Further, we determined the amount of non-structural carbohydrates and the concentration and catalytic activity of Rubisco in order to relate the *in situ* carboxylase activity with the carboxylation efficiency of the stems. While most measurements were made on excised stems brought into the laboratory, we also made measurements on intact stems in the field to assess the effect of the interaction of *in situ* stresses with long-term elevated C_a treatment on photosynthesis.

MATERIALS AND METHODS

Plant material and CO_2 treatments

Monospecific communities of *Scirpus olneyi* growing naturally in a brackish marsh on a subestuary of Chesapeake Bay in Maryland on the Atlantic coast of the United States (38 °N, 76·3 °W) have been exposed using open-top chambers to two levels of C_a throughout the growing season since 1987 in their natural habitat (Drake *et al.* 1989). In this study, the two levels of C_a were the normal ambient concentration (ambient) and the ambient concentration plus 340 μmol mol^{-1} (elevated). The following experiments were conducted on the fully developed (expanded) triangular stems, which are the photosynthetic organs of *Scirpus olneyi*. Measurements were confined to a length of 10–12 cm in the top one-third section of the stem. This section of any given stem contained uniform amounts of soluble proteins and carbohydrates and was not shaded by the plant community. Measurements were expressed on the basis of the surface area of one side of the stem. Previous studies showed that there was no effect of elevated C_a on the morphology or anatomy of the stems (Drake *et al.* 1992).

Gas exchange measurements

Gas exchange measurements were made in the laboratory on excised whole stems and in the field on intact stems with a computerized plant gas exchange system (MPH 1000, Campbell Scientific, Logan, UT) using an infrared gas analyser (Li-6262, Li-COR, Lincoln, NE). For both laboratory and field measurements, we used plants grown at ambient and elevated C_a in the field. We made field and laboratory measurements of gas exchange in order to compare results obtained on stems exposed to the field environment prior to gas exchange measurements with those obtained on stems which did not experience field stress. Laboratory measurements were made on excised stems during September 1993, June 1994, August 1994 and September 1994, and field measurements were made on intact stems in July/August 1992. For the laboratory measurements, stems were cut under water and removed from the open-top chambers early in the morning before the stems experienced any water or heat stress. Gas exchange was measured on these excised stems in the laboratory at a photosynthetic photon flux density (PPFD) of 850–900 μmol m^{-2} s^{-1} incident on the leaf, a leaf temperature of 27 °C and a leaf-to-air vapour pressure deficit (VPD) of 0·9–1·0 kPa. The excised stems, with their cut ends kept in water throughout the measurement, remained fully turgid and showed no visible symptoms of water stress. Gas exchange measurements were made on intact stems in the field between 1000 and 1700 h on warm sunny days. During field measurements, the conditions inside the gas exchange measurement chamber were: PPFD 1450–1600 μmol m^{-2} s^{-1}; leaf temperature 31 °C; leaf-to-air VPD 1·4–1·6 kPa. The leaf internal CO_2 concentration was calculated from steady-state measurements of gas exchange by stems according to Farquhar & Sharkey (1982). Steady-state measurements of A were made at different C_i obtained by varying C_a, and A/C_i response curves were made by fitting an exponential function $[A=a(1-e^{-bC_i})+c]$. From the function, A_{max} was calculated

as $A_{max}=a+c$ and the tissue carboxylation efficiency (defined as the slope of the A/C_i response curve at $A=0$) was calculated as the first-order derivative of the function at $A=0$; $dA/dC_i=(a+c)b$.

Non-structural carbohydrates

The soluble sugar content was determined on the samples used for A/C_i measurements in September 1993 and June 1994. Soluble sugars were extracted in a total volume of 7 cm^3 of 80% (v/v) ethanol from approximately 50 mg fresh weight of the sample (frozen in liquid nitrogen) using a pestle and mortar. The amount of total soluble sugars in the ethanol extract was determined by a modified anthrone method (Cerning-Beroard 1975). A standard curve was prepared with pure glucose, sucrose and fructose dissolved in 80% (v/v) ethanol. The residue obtained after sugar extraction was boiled for 3 min with 5 cm^3 of distilled water and cooled to room temperature, and 150 mm^3 of 2·5 kmol m^{-3} citrate buffer (pH 4·5) was added followed by 100–150 units of amyloglucosidase (reconstituted with 0·05 kmol m^{-3} citrate buffer, pH 4·5). The reaction mixture was incubated overnight at 45 °C to release glucose, the amount of which was determined by the anthrone method. A standard curve was prepared by incubating known amounts of pure starch with amyloglucosidase in the same way as the samples were incubated.

Protein and Rubisco assay

Total soluble protein and Rubisco contents were determined on the stem samples that were used for A/C_i measurements in the laboratory (September 1993 and June 1994). Samples of *Scirpus* stems for analysis were collected and placed in liquid nitrogen immediately after gas exchange measurements in the laboratory or directly from the field. Rubisco was extracted from about 0·1 g fresh weight of the stem samples at 0–4 °C in 3 cm^3 of a freshly prepared buffer solution containing 100 mol m^{-3} bicine-NaOH (pH 8·0), 20 mol m^{-3} $MgCl_2$, 1 mol m^{-3} EDTA (sodium salt), 50 mol m^{-3} mercaptoethanol, 2–3 drops of 40 mol m^{-3} PMSF and 0·02 g acid-washed PVP. The extract was centrifuged for 1 min and the initial specific activity of Rubisco in the supernatant was determined immediately. The initial specific activity is considered to represent the Rubisco activity *in vivo*. The assay was carried out in a modified stoppered O_2 electrode vessel (Hansatech Ltd, Kings Lynn, UK) for 45 s at 25 °C. The assay medium had a final concentration of 100 mol m^{-3} bicine, 20 mol m^{-3} $MgCl_2$, 0·33 mol m^{-3} RuBP, and 10 mol m^{-3} $NaH^{14}CO_3$ (185 kBq per assay), and the initial specific activity was calculated from the amount of ^{14}C fixed into acid-stable products as described by Lorimer, Badger & Andrews (1976). To measure the (Mg^{2+} and HCO_3^- activated) total activity of the enzyme, the extract was preincubated in the assay medium containing no RuBP for 6 min at 25 °C before adding the RuBP to start the reaction. The total activity is considered to represent the maximum potential activity (i.e. when all the sites on the enzyme are fully carbamylated).

In the above crude extract prepared to measure Rubisco, total leaf soluble protein content was determined according to Bradford (1976). The amount of Rubisco in the extract was determined by polyacrylamide gel electrophoresis. The subunits of the enzyme were separated on a 15% SDS-PAGE based on the method of Servaites, Torisky & Chao (1984). Separation was performed at 75 V through the stacking gel and at 100 V through the separating gel, using an electrophoresis power supply unit (BIO-RAD, model 3000/300) for 7 h; the temperature of the gel was maintained at 10 °C using a circulating bath containing ethylene glycol:water (20:80, v/v). After staining the proteins in the gel with Coomassie brilliant blue, the bands corresponding to the subunits of Rubisco were cut and the stain extracted in 1% SDS overnight at 37 °C and absorbance measured at 585 nm. On each gel, separate blanks (tracking dye containing bromophenol blue) and standards (using Rubisco from Sigma Chemicals) were run.

In addition to the samples used for A/C_i measurements in the laboratory (September 1993 and June 1994), fully developed stems were collected directly from the field and frozen in liquid nitrogen, and the amounts of non-structural carbohydrates, soluble proteins and Rubisco were determined. These measurements were made on warm sunny (PPFD=1300 μmol m^{-2} s^{-1}, temperature 32 °C) and cool cloudy (PPFD=185 μmol m^{-2} s^{-1}, temperature 17·5 °C) days. Diurnal changes in the concentration of non-structural carbohydrates were also determined on stem samples collected directly from the field. The above measurements on samples collected directly from the field were made in July/August 1993.

RESULTS

Gas exchange measurements in the laboratory

The response of photosynthesis to C_i measured on excised stems in the laboratory between September 1993 and September 1994 was altered by long-term elevated C_a treatment (Fig. 1). A clear reduction in the initial slope of the A/C_i response and an increase in A (when compared at growth C_a, indicated by arrows in Fig. 1) were evident in elevated-C_a-grown *Scirpus* compared with ambient-C_a- grown plants.

The photosynthetic rate at the growth C_a was always significantly greater (30 to 59%) in *Scirpus* stems grown at elevated C_a than in those grown at ambient C_a in all the measurements made between September 1993 and September 1994 (Fig. 2a). However, when compared at the normal ambient C_a, plants grown at elevated C_a had significantly lower A (40 to 52%) than those grown at normal ambient C_a (Fig. 2a). The carboxylation efficiency (defined as the initial slope of the A/C_i response curve at $A=0$) was lower (22 to 45%) in plants grown at elevated C_a than in plants grown at ambient C_a (Fig. 2b). The reduction in carboxylation efficiency was statistically significant, except

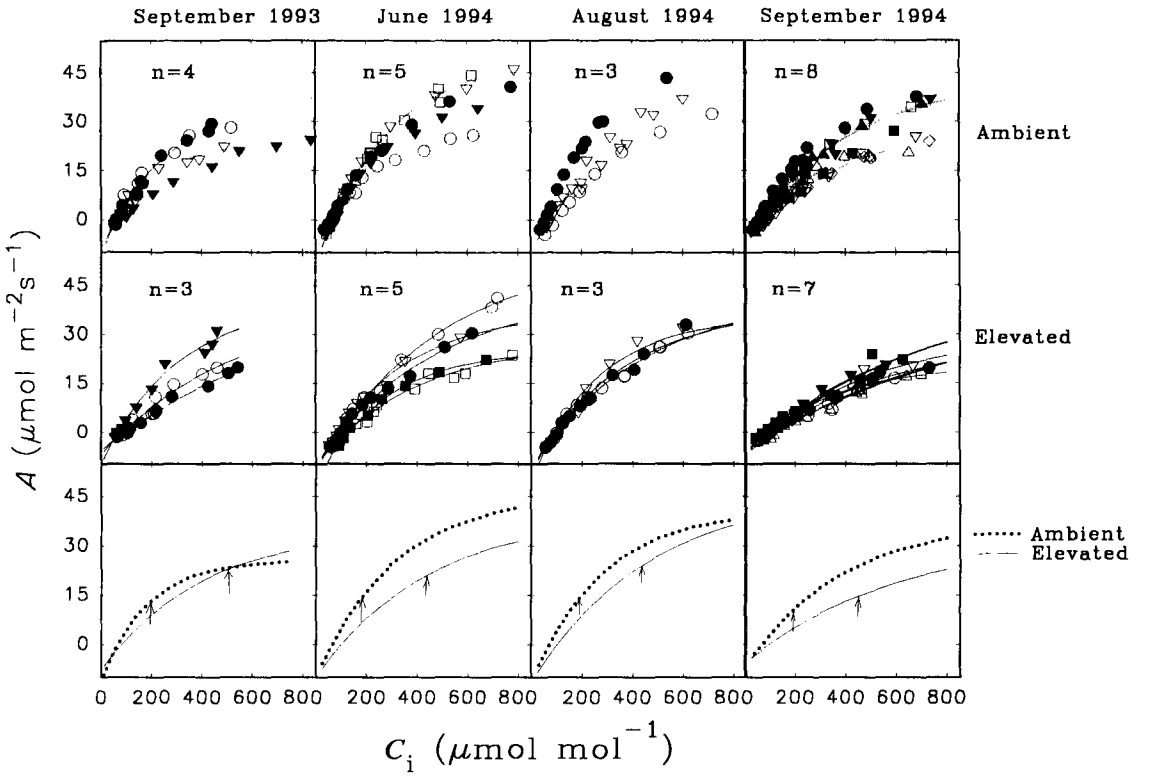

Figure 1. A/C_i response curves determined on excised stems of *Scirpus* plants grown at ambient (top panels) and elevated (middle panels) C_a in the field during September 1993, June 1994, August 1994 and September 1994. Each symbol represents one independent sample. The dotted and solid curves in the bottom panels represent the fitted A/C_i response functions of all the ambient- and elevated-C_a-grown stems represented in the top and middle panels, respectively. The curves are of the form $A=a(1-e^{-bC_i})+c$ and the R^2 range from 89 to 99%.

in August 1994 (Fig. 2b). A_{max} was significantly higher in elevated-C_a-grown plants in September 1993, but not in 1994 (Fig. 2c).

Gas exchange measurements in the field

Figure 3 shows a summary of the results of gas exchange measurements made on intact stems in the field. When measurements were made in the field at the growth C_a, stems grown at elevated C_a had significantly higher (100%) A than stems grown at ambient C_a. Similarly, A_{max} was significantly higher (60%) in elevated-C_a-grown stems than in ambient-C_a-grown stems. The carboxylation efficiency showed no statistically significant reduction with elevated C_a. All values of A, A_{max} and carboxylation efficiency determined on plants grown in normal ambient C_a were lower when measured on intact stems in the field than when measured on excised stems used in the laboratory. This suggests that the field environment was more stressful than the laboratory environment.

Tissue composition of samples from the laboratory gas exchange measurements

On the stems that were used to study the A/C_i responses in the laboratory in September 1993 and June 1994, we measured the contents of soluble sugars, starch, soluble proteins and Rubisco (Table 1). Total soluble sugars increased by 51 to 52% and starch increased by 44 to 54% in the elevated-C_a-grown stems. In these same samples, there was a significant reduction in the total soluble protein (39%) and Rubisco (30 to 58%) contents caused by elevated C_a. The content of Rubisco relative to total soluble proteins was 45 to 46% in ambient and 43 to 53% in elevated-C_a-grown stems. There was a positive linear correlation (r=0·82, n=17) between the Rubisco content in the stems and their carboxylation efficiency (Fig. 4).

Tissue composition of samples taken directly from the field

Total soluble proteins and Rubisco

In *Scirpus* stems sampled directly from the field on a warm and sunny day, elevated C_a significantly decreased the content of total soluble protein and Rubisco per unit stem area (Fig. 5a). The content of Rubisco relative to that of total soluble proteins was not markedly altered by the change in growth C_a (58% in ambient C_a and 54% in elevated C_a). There was no significant effect of growth C_a on the initial specific activity or total activity of Rubisco expressed on a Rubisco basis on the warm and sunny day (Fig. 5b). The activation state of Rubisco in the elevated-C_a-grown *Scir-*

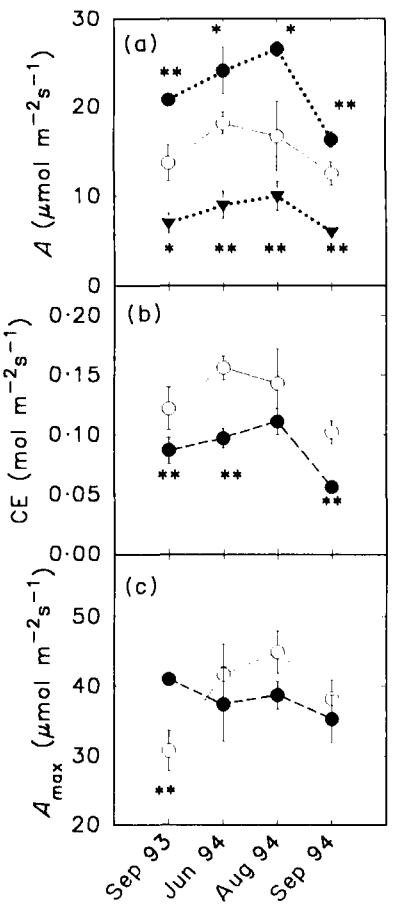

Figure 2. Summary of gas exchange measurements made in the laboratory on excised *Scirpus* stems grown at normal ambient (open symbols) and elevated (closed symbols) C_a. In (a) the photosynthetic rates of ambient-C_a-grown (open circles) and elevated-C_a-grown (closed circles) *Scirpus* stems measured at their respective growth C_a are shown. Shown in the same panel are the photosynthetic rates of elevated-C_a-grown *Scirpus* stems measured at normal ambient C_a (closed triangles). * and ** positioned above the closed circles are for a comparison of the open and closed circles and those positioned below the closed triangles are for a comparison of the open circles with the closed triangles. (b) shows the carboxylation efficiency (CE) and (c) shows A_{max} of *Scirpus* stems grown at ambient (open circles) and elevated (closed circles) C_a. Measurements were made in September 1993 (n=3–4), June 1994 (n=5), August 1994 (n=3) and September 1994 (n=7–8). * indicates significance in an independent t-test at P<0·07 and ** indicates significance in an independent t-test at P<0·05.

pus stems increased slightly compared to the normal ambient-C_a-grown stems, but the increase was not significant (Fig. 5b). The *in situ* carboxylase activity of the stems calculated from the Rubisco content and its initial specific activity decreased by 43% in plants grown at elevated C_a compared to those grown at ambient C_a (Fig. 5c).

The initial specific activity or the total activity of Rubisco (expressed on a Rubisco basis) did not show any effect of growth C_a when studied in *Scirpus* stems collected on a cool and cloudy day (Fig. 5d). Compared to the warm and sunny day, the initial specific activity and activation state of Rubisco were much lower on the cool and cloudy day; however, the total activities of Rubisco were similar on the two days.

Carbohydrates

Figure 6 shows soluble sugar and starch contents determined on *Scirpus* stems collected directly from the field at three times on a warm and sunny day. At 0800 and 2000 h, stems at elevated and ambient C_a contained similar amounts of soluble sugars, but at 1400 stems grown at elevated C_a had significantly higher amounts of soluble sugars. The starch content was 18 and 19% greater in stems at elevated C_a than in stems at ambient C_a when measured at 0800 and 1400 h, respectively (Fig. 6). At 2000 h, there was no significant difference in the amount of starch in the stems. Soluble sugar and starch contents were significantly greater in *Scirpus* stems grown at elevated C_a than in those grown at ambient C_a when determined on samples collected directly from the field around midday on a warm and sunny day, but not on a cool and cloudy day (data not shown).

DISCUSSION

Photosynthesis

Scirpus plants grown at elevated C_a in the field for the past 8 years sustained a high photosynthetic rate (A) compared with those grown in normal ambient C_a when measurements were made at their respective growth C_a. This increase was 30 to 59% when measured on excised stems in the laboratory (Fig. 2a) and 100% when measured on intact stems in the field (Fig. 3). Similar results were previously obtained for *Scirpus* (Zizka, Drake & Chamberlain

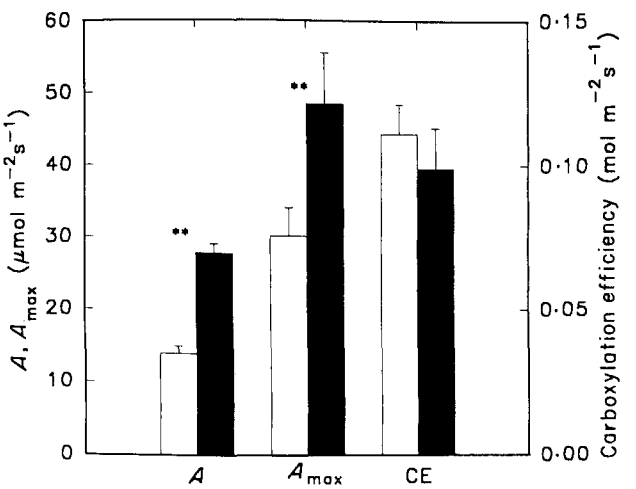

Figure 3. A, A_{max} and carboxylation efficiency (CE) of ambient-(open bars) and elevated-(solid bars) C_a-grown *Scirpus* stems measured in the field in July/August 1992. * indicates a significant independent t-test at P<0·07 and ** indicates a significant independent t-test at P<0·05.

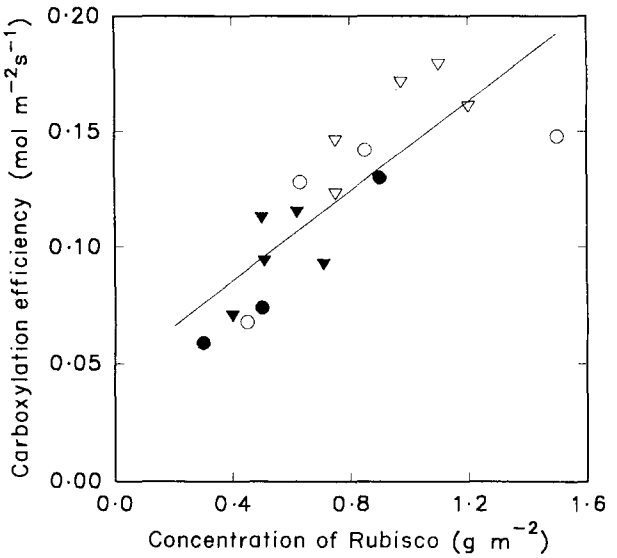

Figure 4. Correlation between Rubisco concentration and carboxylation efficiency determined on excised stems of *Scirpus* from ambient (open symbols) and elevated (closed symbols) C_a (r=0·82; n=17). Circles represent the samples used for A/C_i measurements in September 1993 and triangles represent the samples used for A/C_i measurements in June 1994.

1990; Arp & Drake 1991) and other native species such as white oak, yellow poplar (Wullschleger, Norby & Hendrix 1992; Gunderson, Norby & Wullschleger 1993), *Populus grandidentata* (Curtis & Teeri 1992), loblolly pine (Tissue *et al.* 1993), *Ranunculus glacialis* (Körner & Diemer 1994) and *Paseopyrum smithii* (Morgan *et al.* 1994), but not for C_3 crop species such as *Chenopodium album*, *Phaseolus vulgaris* and *Brassica oleracea* (Sage *et al.* 1989) and cotton (Thomas & Strain 1991). When compared at the normal ambient C_a, A was 40–52% lower in plants grown at elevated C_a than in those grown at ambient C_a (Fig. 2a). This was largely a result of a reduced concentration of Rubisco in plants grown at elevated C_a (Table 1 & Fig. 5).

The photosynthetic rate (13–28 μmol m^{-2} s^{-1}) and Rubisco content (0·6–1·0 g m^{-2}) were lower in *Scirpus* stems than in C_3 crop plants grown with adequate fertilizers, but greater than in some native C_3 species. For instance, in healthy sunflower leaves grown at normal ambient C_a, A and Rubisco content ranged from 30–35 μmol m^{-2} s^{-1} and from 3 to 4 g m^{-2}, respectively (Jacob & Lawlor 1991, 1992). In an experiment with five different C_3 crop species grown at normal ambient C_a, A ranged from 20 to 35 μmol m^{-2} s^{-1} and the Rubisco content ranged from 1·6 to 3·0 g m^{-2} (Sage *et al.* 1989). On the other hand, tree species had lower rates of photosynthesis and Rubisco content: in loblolly pine, A was 1–5 μmol m^{-2} s^{-1} and the Rubisco content was 0·03–0·22 g m^{-2} (Tissue *et al.* 1993), and in *Populus grandidentata*, A ranged from 10 to 20 μmol m^{-2} s^{-1} depending on the growth C_a (Curtis & Teeri 1992). The photosynthetic rate measured in previous years of CO_2 enrichment in *Scirpus* ranged from 7 to 32 μmol m^{-2} s^{-1} depending on the background C_a, light and temperature (Ziska *et al.* 1990; Arp & Drake 1991). Although A and the Rubisco content were lower in *Scirpus* than in some crop species, the amount of Rubisco relative to total soluble proteins was similar to that found in other C_3 species (Woodrow & Berry 1988; Jacob & Lawlor 1992; Tissue *et al.* 1993).

Laboratory versus field measurements of photosynthesis

Growth at elevated C_a resulted in greater stimulation of A and A_{max} when measurements were made on intact stems in the field than when they were made on excised stems in the laboratory (Figs 2 & 3 and Arp & Drake 1991). Stems used for gas exchange measurements in the laboratory were cut from the field early in the morning before any abiotic stress, such as high PPFD or temperature, could set in. Various interacting environmental factors in the natural environment can confound the effects of elevated C_a on gas exchange data and make the interpretation of the data from field and laboratory measurements difficult. Drought stress (Chaves & Pereira 1992), high temperature (Long 1991; Long & Drake 1992) and high light (Ziska *et al.* 1990; Curtis & Teeri 1992) lead to maintenance of higher rates of photosynthesis in plants growing at elevated C_a

Table 1. Tissue composition of *Scirpus* stems used for A/C_i response measurements in the laboratory during September 1993 (n=3–4) and June 1994 (n=5). (All values are expressed in g m^{-2}. Figures in parentheses are standard errors of the means. * = significant independent t-test at P<0·07; ** = significant independent t-test at P<0·05.)

	September 1993				June 1994			
Treatment	Soluble Sugar	Starch	Soluble Protein	Rubisco	Soluble Sugar	Starch	Soluble Protein	Rubisco
Ambient	12·9 (0·9)	60·7 (5·7)	1·80 (0·34)	0·835 (0·148)	19·7 (2·33)	51·3 (6·17)	2·11 (0·20)	0·954 (0·091)
Elevated	19·5 (4·0)	87·4 (8·1)	1·10 (0·06)	0·584 (0·085)	29·8 (3·12)	79·0 (4·73)	1·28 (0·14)	0·550 (0·053)
Change from ambient (%)	+52	+44	−39	−30	+51	+54	−39	−58
t test	**	**	*	*	**	**	**	**

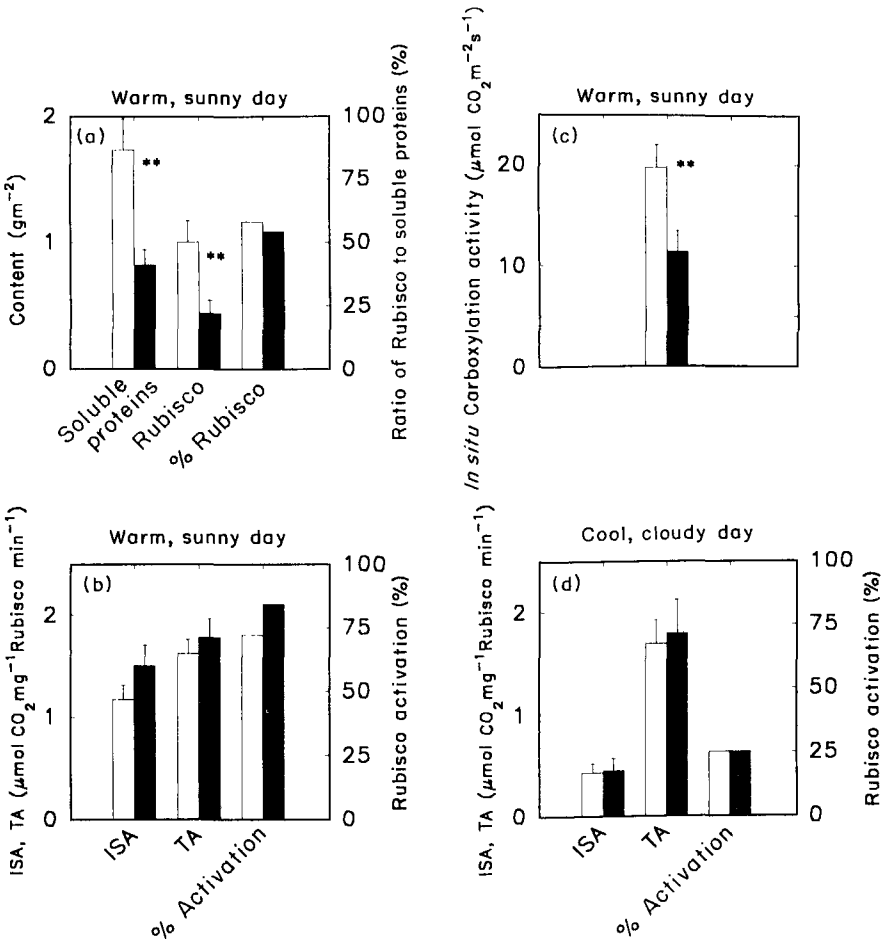

Figure 5. The effect of long-term CO_2 enrichment on the contents of soluble protein and Rubisco (a) and the initial specific activity (ISA), the total activity (TA) and the activation state of Rubisco (b,d) determined on a warm, sunny day (a,b) and on a cool, cloudy day (d) and the *in situ* carboxylase activity determined on the warm, sunny day (c) in *Scirpus* stems. The mean PPFD and air temperature just above the plants determined from measurements made at 15 min intervals between sunrise and the sampling time (between 1000 and 1100 h) were, respectively, 1300 μmol m^{-2} s^{-1} and 32 °C for the warm, sunny day and 185 μmol m^{-2} s^{-1} and 17·5 °C for the cool, cloudy day. Open bars indicate plants grown at ambient C_a and solid bars indicate plants grown at elevated C_a. * indicates a significant independent *t*-test at $P<0.07$ and ** indicates significant independent *t*-test at $P<0.05$.

than in those growing at ambient C_a. In this study, field measurements with intact stems were made at a higher PPFD, leaf-to-air VPD and temperature than laboratory measurements with excised stems. These differences in the abiotic and biotic factors during gas exchange measurements may help to explain the greater stimulation of A and A_{max} observed in elevated-C_a-grown plants when measured in the field. It should also be noted that, in spite of a significant reduction in the Rubisco concentration in plants grown at elevated C_a (Fig. 5), the reduction in carboxylation efficiency was not statistically significant for measurements on intact stems in the field (Fig. 3). Arp & Drake (1991) obtained lower rates of photosynthesis and found that there was no statistically significant effect of growth C_a on carboxylation efficiency in *Scirpus* when measurements were taken on intact stems in the field on several afternoons in August 1990. Field-grown plants measured under field conditions, particularly during hot afternoons, probably experience stresses which modify the direct effects of elevated C_a on gas exchange. We speculate that various interacting environmental conditions in the field could confound the effects of CO_2 on carboxylation efficiency and the absolute rates of photosynthesis. When measurements are made under field conditions, abiotic stresses such as high temperature and physiological drought will have a greater inhibitory effect on carboxylation efficiency and other gas exchange parameters in plants grown at ambient C_a than in those grown at elevated C_a (Figs 2&3). As a consequence, plants grown at normal ambient C_a will have lower rates of photosynthesis and carboxylation efficiency in the field than in the laboratory.

Soluble proteins and Rubisco

Growth at elevated C_a decreased the total soluble protein and Rubisco contents in *Scirpus* stems, but did not alter the

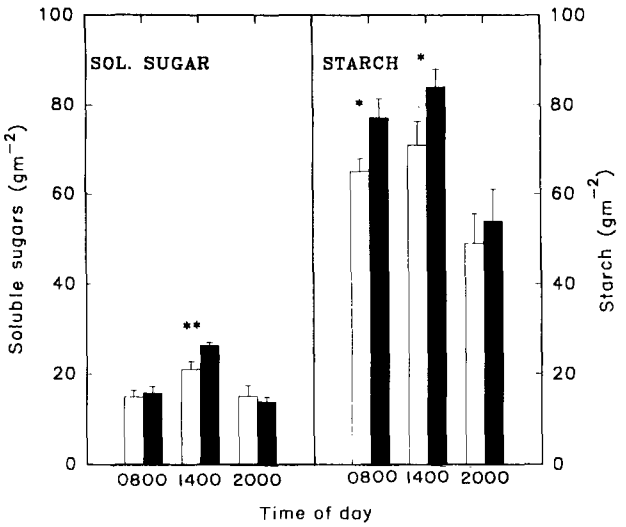

Figure 6. Amounts of soluble sugars and starch in *Scirpus* stems grown at ambient (open bars) and elevated (solid bars) C_a in the field determined at 0800, 1400 and 2000 h on a warm, sunny day. The mean PPFD and air temperature just above the plants determined from measurements made at 15 min intervals between 1315 and 1415 h were, respectively, 1850 μmol m^{-2} s^{-1} and 33 °C, and between 1915 and 2015 h they were 30 μmol m^{-2} s^{-1} and 24 °C. PFD and temperature data were not available for the measurement made at 0800 h. * indicates a significant independent *t*-test at $P<0.07$ and ** indicates a significant independent *t*-test at $P<0.05$.

Rubisco activation state when measurements were made in stem samples collected in the field on a warm sunny day (Table 1 & Fig. 5). On a cool cloudy day, the initial specific activity and activation state of Rubisco were markedly reduced compared to the warm sunny day, illustrating the dominant regulatory effect of light intensity on Rubisco activity (Fig. 5). The tight binding inhibitor, 2-carboxy arabinitol 1-phosphate (CA-1-P), which is known to accumulate at very low light intensities in some species, does not appear to be involved in reducing the Rubisco activity in *Scirpus* exposed to low light intensity because the total activity of Rubisco from *Scirpus* sampled on the cloudy day was nearly as large as that on the sunny day. The above results on Rubisco agree with the findings of Tissue *et al.* (1993) in loblolly pine needles. However, Sage *et al.* (1989) found that elevated C_a decreased the Rubisco content in some species, but decreased Rubisco activation state significantly in all the five C_3 crop species they studied.

In situ carboxylase activity was positively correlated with the initial slope (carboxylation efficiency) of the A/C_i response curves (Fig. 4). The decrease in the *in situ* carboxylase activity (Fig. 5c) in elevated-C_a-grown *Scirpus* stems resulted in a lower carboxylation efficiency compared to stems grown at ambient C_a (Fig. 2b). The decreased amount of Rubisco was responsible for the decrease in the *in situ* carboxylase activity in plants grown at elevated C_a (Figs 5a&c). Reduced carboxylase activity with elevated C_a has been observed in a large number of studies, and this reduction ranged from 10 to 50% depending on the species and growth conditions (Stitt 1991).

Rubisco is the single largest sink for organic N in photosynthesizing tissues, often accounting for 12–23% of the total N of a C_3 leaf. Consequently, a reduction in its content will contribute more to the reduction in the tissue N concentration than a reduction in the content of any other single protein. Elevated-C_a-reduced the soluble protein and N concentrations in leaves of C_3 plants such as cotton, but not in leaves of C_4 plants such as maize (Wong 1979), *Spartina patens* or *Distichlis spicata* (Curtis *et al.* 1989). Elevated C_a grown *Scirpus* (Curtis, Drake & Whigham 1989) and other native species such as *Rumex obtusifolius* (Diaz *et al.* 1994) had significantly lower N concentrations. As a consequence of increased photosynthesis and decreased tissue N concentration, the photosynthetic N use efficiency increased in elevated-C_a-grown *Scirpus*.

Carbohydrates

The increased photosynthetic response to elevated C_a led to a significant increase (25 to 53%) in the content of non-structural carbohydrates in *Scirpus* stems (Table 1). The amounts of soluble sugars and starch found in the *Scirpus* stems grown at elevated C_a, which were higher than those found in stems during the day, declined to similar levels at 2000 h (Fig. 6), suggesting that the sink demand in the remainder of the plant could keep pace with increased production of photosynthates in *Scirpus* stems grown at elevated C_a in the field. It appears that, in the *Scirpus* plants in the present investigation grown at elevated C_a in the field as well as in other native species grown at elevated C_a with unrestricted rooting volume, the sink capacity of the plant was large enough to utilize the newly formed photosynthates in the source leaves, and the rate of photosynthesis was maintained at a high level when compared with ambient-C_a-grown plants at growth C_a concentrations. The starch content increased by 22% in loblolly pine needles (Tissue *et al.* 1993) and by 70% in leaves of yellow poplar and white oak (Wullschleger *et al.* 1992) in response to elevated C_a. In another native species, *Ranunculus glacialis*, the non-structural carbohydrate content increased by 100% in response to elevated C_a with no effects on the A/C_i response curves (Körner & Diemer 1994). In the above studies and the present investigation, in spite of a significant increase in the amount of carbohydrates, A was greater in plants growing at elevated C_a than in plants growing at ambient C_a when plants were compared at their respective growth C_a.

CONCLUSIONS

After eight years of growth at elevated C_a in the field, the rate of photosynthetic CO_2 assimilation of the *Scirpus* stems was maintained at levels 30–100% higher in plants

grown in elevated C_a than in those grown in ambient C_a *when plants were compared at their respective growth* C_a, although the concentration of Rubisco was about 30–58% lower in stems grown at elevated C_a. Long-term elevated C_a treatment in the field decreased the Rubisco content, the *in situ* carboxylase activity and carboxylation efficiency, which resulted in lower CO_2 assimilation rates in plants grown at elevated C_a than in those grown at ambient C_a *when plants were compared at normal ambient* C_a. Plants grown at elevated C_a contained more non-structural carbohydrates than those grown at normal ambient C_a. Our results are in overall agreement with the hypothesis that elevated C_a leads to an increased carbohydrate concentration and the ensuing acclimation of the photosynthetic apparatus in C_3 plants results in a reduction in the protein complement, especially Rubisco. Alterations in the biochemical composition of the tissue in response to continuous growth at elevated C_a will have consequences for physiological (e.g. respiration) and ecological (e.g. decomposition, herbivory) processes.

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