CO₂ Enrichment of Greenhouse Roses Affects Neither Rubisco Nor Carbonic Anhydrase Activities

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Abstract. The effect of prolonged CO₂ enrichment on the activities of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and carbonic anhydrase (CA) of greenhouse roses were studied. Plants of *Rosa* × *hybrida* 'Red Success' were grown for 2 years at ambient and 900 µl CO₂/liter during winter and spring with 75 µmol·m⁻²·s⁻¹ photosynthetically active radiation supplemental lighting for 2 years. Measurements of initial and Mg²⁺-CO₂-activated activities of Rubisco and CA were made during shoot development and at different positions within the plant canopy. Generally, there were no significant differences measured in the enzyme activities between the two CO₂ concentrations. The results suggest that the photosynthetic capacity did not change and that there were no characteristic adaptations to long-term growth (up to 20 weeks) at elevated CO₂ concentrations. The maintenance of Rubisco and CA activities with prolonged exposure to CO₂-enriched atmospheres is proposed as the reason for long-term yield increases in roses when grown in enriched environments.

Enrichment of greenhouse atmospheres with CO₂ results in increased yields of roses of 16% to 60% over those grown in a nonenriched atmosphere (Enoch et al., 1970; Hanan, 1973; Mortensen and Moe, 1983). In nonwoody species, such as tomato (Besford et al., 1985; Hincklenton and Jolliffe, 1980; Yelle et al., 1987), cucumber (Peet et al., 1986), chrysanthemum (Mortensen, 1984), and water hyacinth (Spencer and Bowes, 1986), CO₂ enrichment initially increases photosynthesis, growth, and yield. However, both photosynthesis and growth rates diminished with extended CO₂ enrichment. This decline in photosynthesis has generally been associated with lower ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Peet et al., 1986; Porter and Grodzinski, 1984; Spencer and Bowes, 1986; Yelle et al., 1989). Lower stomatal conductance (Spencer and Bowes, 1986) and decreases in CA activities (Porter and Grodzinski, 1984) have also been reported. The decrease in photosynthesis results in similar photosynthetic rates between plants in the enriched environments and their ambient-grown counterparts, provided the measurements are made at the CO₂ concentrations in which the plants were grown. When measured under ambient concentrations, plants previously grown under enriched conditions have lower photosynthetic rates (Frydrych, 1976).

Investigations of the physiological effects of long-term CO₂ enrichment on semiwoody and woody species are rare. Balazsovits et al. (1989) reported enhanced yields of up to 60% over control roses during 6 months of enrichment, with diminishing enhancement continuing for up to 2 months after enrichment was stopped. This result suggests that roses do not acclimatize to long-term CO₂ enrichment and, thus, maintain the initial growth rates. In an effort to understand this contradiction with reported effects of prolonged CO₂ enrichment on other species, we studied the effects of CO₂ enrichment on the activities of Rubisco

and CA during stem development and as a function of leaf position on harvestable stems. The objective was to determine if long-term CO₂ enrichment results in a decline in Rubisco or CA activity of greenhouse roses.

Materials and Methods

Experiments were performed on containerized roses at the greenhouse complex at Laval Univ. in Quebec City (lat. 49°). Plants of the hybrid tea *Rosa* × *hybrida* 'Royalty' had been grown previously for 1 year in 30-liter polypropylene containers in a 60 sand : 40 peat (v/v) mixture. The experiment was designed as a split plot, using CO₂ treatment as the main plot and either canopy position or time as the subplot. One leaf was sampled per repetition and there were four repetitions per subplot. Plants were fertilized with a commercial mixture of 20N-2.1P-9.9K plus micronutrients alternating with 20N-8.7P-11K and 15N-7.0P-9.3K (Plant Products, St. Catherine, Ont., Canada) three out of every four irrigations. Before the experiment, the plants were grown under ambient atmospheres. During Expt. 1, plants were grown in two identical, adjacent compartments in a glass greenhouse under noncontrolled ambient CO₂ (340 ± 70 µl·liter⁻¹, or 900 ± 100 µl CO₂/liter from 22 Dec. 1987 to 10 May 1988. Carbon dioxide concentrations were measured using a Priva CO₂ controller (Priva, Holland). The same controller maintained the CO₂ concentrations in the enriched compartment by injection of CO₂ from a gas cylinder. Plants were grown with 30 cm between containers and 20 plants in each 5-m² compartment. Temperatures within each compartment were controlled independently and maintained at 18 to 22°C/16°C (day/night). Relative humidities were similar for the treatments and low (10% to 20%) during the day until April. Plants received 75 µmol·m⁻²·s⁻¹ photosynthetically active radiation from (PAR) supplemental lighting by high-pressure sodium lamps (HPS) at 1.5 m above the greenhouse floor during overcast days and at

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Abbreviations: CA, carbonic anhydrase; PAR, photosynthetically active radiation.

night to extend the photoperiod to 24 h. All plant shoots had been cut to 25 cm above the soil in early Dec. 1987. When flowering shoots reached commercial maturity, they were removed at two nodes above the previous cut. Commercial maturity was defined as the time when exterior petals began to unfold. Five to 7 weeks were required for a flowering stem to reach commercial maturity when initiated from a previously harvested stem. The experiment was repeated in 1989 (Expt. 2) with the same plants grown under similar conditions from 1 Mar. to mid-May. The plants had been pruned to 25 cm in July 1988 and then grown under ambient CO_2 atmospheres until the start of Expt. 2.

The effects of CO_2 enrichment at different positions within the canopy were evaluated during both experiments by sampling single leaflets taken from three (Expt. 2) or four (Expt. 1) evenly spaced positions above the last cut on a stem just before harvest. Stems averaged 79 ± 15 cm, with sampling extending from the uppermost leaf to the bottom leaf. This procedure was repeated on harvestable stems 7, 11, 14, and 20 weeks after the start of CO_2 enrichment in Expt. 1 and after 4 and 6 weeks in Expt. 2. Four shoot replicates per CO_2 treatment were taken per sampling. The terminal leaflet of the selected leaf was cut and immediately plunged into liquid nitrogen. Samples were stored at -80°C until the enzyme was extracted.

Changes in enzyme activities during shoot development were followed on the oldest pentfoliate leaf developing on a new stem. Sampling began when the leaflets on the first five-leaflet leaf were one-fourth to one-half fully expanded. This corresponded to the previsible bud stage. Weekly thereafter, a leaflet was taken from the same leaf as described previously. Sampling was replicated on four developing shoots per CO_2 treatment. The effect of CO_2 enrichment in Expt. 1 were measured during two periods of shoot development on two sets of developing shoots: weeks 11 to 14 and 16 to 20 after the CO_2 enrichment was started. In Expt. 2, measurements were made during the periods of 2 to 5 weeks and 6 to 9 weeks after the start of enrichment.

Rubisco (E.C. 4.1.1.39) activities were assayed as described by Beeson (1990). Briefly, samples were ground in liquid nitrogen then homogenized with the extraction solution (100 mM Bicine, 5 mM sodium ascorbate, 5 mM dithiothreitol (DTT), and 1.0 mM NaEDTA, pH 8.1). One milliliter was removed for determination of chlorophyll content (Arnon, 1949), while 7 ml was centrifuged at $15,000 \times g$ for 5 min at 2°C . One milliliter of the supernatant was combined with 1 ml of activation solution (100 mM Bicine, 20 mM MgCl_2 , and 20 mM NaHCO_3 , pH 8.1) and incubated at 20 to 22°C for 20 min before being used to assay for the Mg^{2+} - CO_2 -activated activity. Measurement of the initial Rubisco rates was made on the supernatant.

The assay solution constituted 100 mM Bicine, 5 mM dithiothreitol, 0.4 mM ribulose 1,5 biphosphate, 0.1 mM sodium ethylenediaminetetraacetic acid (NaEDTA), 20 mM MgCl_2 , and 10 mM $\text{NaH}^{14}\text{CO}_3$ [10 mmol/mCi (1 Ci = 37 GBq); pH 8.1] at a final volume of 1.0 ml. The assay ran for 2 min at 25°C and was stopped by the addition of 65 μl 6 N HCl. The vials were then dried and counted with a liquid scintillation counter. Two aliquots of each sample were assayed.

CA (E.C. 4.2.1.1) activities were measured on the same crude extract as initial Rubisco using an adaptation of the method of Bruns et al. (1986). The assay solution consisted of 0.5 ml of the indicator solution (2.5 mg phenol red in 100 ml 5 mM NaHCO_3), 50 μl 250 mM NaHCO_3 (pH 8.2), and 0.8 ml of the Bicine extract solution mixed in 10-ml glass test tubes. Solu-

Fig. 1. Initial Rubisco activities in relation to position in the rose canopy after 7 (A) and 20 (B) weeks of growth at ambient CO_2 (□) or with 900 μl CO_2 /liter (■) (Expt. 1). Position 1 corresponds to the uppermost leaf of the harvestable cane. Each mean represents four leaves. The vertical bars indicate the standard error of the means.

tions were maintained at 0°C . For each assay, 0.15 ml of the supernatant was pipetted into a test tube and mixed with the assay solution. Immediately thereafter, the assay was started by the addition of 1.0 ml of CO_2 -saturated (CO_2 bubbled for a minimum of 1 h), distilled deionized water to the test tube. The time required to reach a yellow endpoint was recorded for each tube. Three aliquots of each sample were assayed with the endpoint determined visually by comparison with a previously assayed aliquot. CA activity was calculated using the equation of Wilbur and Anderson (1948), where: units CA = $[10 \times (\text{time without extract} / \text{time with extract})] - 1$. All assays were normalized to CA units per milligram of chlorophyll.

Analysis of variance using a split-plot design was calculated for each canopy position/harvest and canopy development period. Canopy position studies were analyzed using CO_2 treatments as the main plot and position the subplot. Developmental periods were analyzed as repeated measurements (Snedecor and Cochran, 1980), with CO_2 treatment as the main plot and time as the subplot. Regression analysis of the shoot development periods were considered but not used due to variability in solar irradiances between sampling periods. Though sampling always occurred in early afternoon, solar irradiances between samplings ranged from 250 to 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR. Means within subplots were separated by protected LSDs when the main plot and subplot interaction was significant (Snedecor and Cochran, 1980).

Canopy position. Initial Rubisco activities of leaflets from plants grown at noncontrolled CO_2 concentrations in Expt. 1

Fig.2. Initial (A, B) Rubisco activities and the activities of CA (C, D) in relation to position within arrose canopy (Expt.2). Activities were measured after 4 (A and C) and 6 (B and D) weeks of growth at ambient CO_2 (□) and $900 \mu\text{l CO}_2/\text{liter}$ (■). Position 1 corresponds to the uppermost leaf of the harvestable cane. Each mean represents four leaves. The vertical bars indicate the standard errors of the means.

were generally higher than those grown at $900 \mu\text{l-liter}^{-1}$ through week 14. At the 7- and 14-week harvests, the interaction of CO_2 treatment and position was significant ($\alpha = 0.05$), but not at the 11-week harvest. Differences between CO_2 treatments within a position were only significant for the uppermost leaf (position 1), as represented by the 7-week harvest (Fig. 1A). Differences between positions, averaged over CO_2 treatments, were always significant, with the activities declining from the uppermost leaf (position 1) to the bottom leaf.

No differences in activity were found in the $\text{Mg}^{+2}\text{-CO}_2$ activated form of Rubisco, regardless of canopy position (range $150\text{-}200 \mu\text{mol CO}_2/\text{mg chlorophyll per h}$) through week 14. However, leaflets sampled from plants grown under noncontrolled CO_2 conditions tended to have higher activities than CO_2 -enriched plants at weeks 11 and 14 (data not shown). This trend was not evident for leaves from shoots harvested at week 7.

At the 20-week harvest, initial activities were similar between positions within a treatment (Fig. 1B). In contrast to earlier weeks, plants grown at $900 \mu\text{l-liter}^{-1}$ had Rubisco activities significantly ($\alpha = 0.05$) higher than those under noncontrolled conditions. The $\text{Mg}^{+2}\text{-CO}_2$ -activated activities were again similar for treatments and canopy positions (range $140\text{-}150 \mu\text{mol CO}_2/\text{mg chlorophyll per h}$).

In Expt. 2, "plants that received CO_2 at $900 \mu\text{l-liter}^{-1}$ had significantly higher initial Rubisco activities than the controls at the first harvest (week 4; Fig. 2A), but not the second (week 6; Fig. 2B). There were no differences within canopy positions. In neither harvest were differences found in the $\text{Mg}^{+2}\text{-CO}_2$ -activated rates (range $250\text{-}325 \mu\text{mol CO}_2/\text{mg chlorophyll per h}$, harvest 1, and $280\text{-}430 \mu\text{mol CO}_2/\text{mg chlorophyll N per h}$, harvest 2). CA activities were similar for treatments and harvest

(Fig. 2 C and D), but declined significantly with increasing canopy depth (increasing position number).

Shoot development. Differences between CO_2 treatments in Rubisco activity during shoot development depended on the period of sampling in Expt. 1. During the first development period, weeks 11 to 14, plants grown in the noncontrolled compartment had significantly ($\alpha = 0.05$) higher initial activities than those grown at $900 \mu\text{l-liter}^{-1}$ (Fig. 3A). Significant differences between CO_2 treatments in $\text{Mg}^{+2}\text{-CO}_2$ -activated activities depended on leaf age, with those of ambient-grown plants becoming higher than those of enriched plants during the last 2 weeks before commercial harvest (Fig. 3B).

In contrast to the first period, initial activities were not significantly different between CO_2 treatments during weeks 16 to 20 (Fig. 4A). However, similar to the first period, they were significantly affected by leaf age. The interaction between CO_2 treatment and leaf age was significant for $\text{Mg}^{+2}\text{-CO}_2$ -activated rates (Fig. 4B). However, there were no consistent differences between treatments over the two periods.

Differences in initial activities were not significant between treatments in Expt. 2 and were only affected by time during the first development period, weeks 2 to 5 (Fig. 5A). During the second period, weeks 6 to 9, the CO_2 treatment $\times$ time interaction was significant ($\alpha = 0.05$). Yet, only during week 9 were the initial activities higher for the CO_2 -enriched plants (Fig. 5B). Measured $\text{Mg}^{+2}\text{-CO}_2$ -activated rates were significantly affected by leaf age during weeks 2 to 5, while the significant differences during weeks 6 to 9 depended on both time and treatment (Fig. 5 C and D). CA activities of plants grown in the noncontrolled compartment were significantly higher than those of plants in the enriched atmosphere during weeks 2 to 5 (Fig. 5E), but no treatment effect was found during the second

Fig. 3. Changes in (A) initial and (B) Mg^{2+} - CO_2 -activated Rubisco activities for plants grown in ambient CO_2 (O) or $900 \mu\text{l CO}_2/\text{liter}$ (●) during the development of flowering shoots for weeks 11 to 14 (Expt. 1). Each point represents four leaves. The vertical bars indicate the standard errors of the means.

period (Fig. 5F). During the second period, CA activities declined significantly with time.

Discussion

Rubisco is the principal enzyme in CO_2 assimilation in plants and exists in two functional states (Perchorowicz et al., 1981). The initial state is considered to reflect the actual rate of carbon assimilation under the sampling conditions (Seeman and Berry, 1981), while the Mg^{2+} - CO_2 -activated state can be thought of as the enzyme capacity under optimum conditions (Lorimer et al., 1976). A strong linear correlation existed between initial Rubisco activity and net photosynthesis in greenhouse rose leaves (Beeson, 1990); thus, measurements of initial activity provide an estimate of net photosynthetic rates. Measurements of the Mg^{2+} - CO_2 -activated activities estimate maximum photosynthetic rates and the quantity of functional Rubisco in a leaf.

Fig. 4. Changes in (A) initial and (B) Mg^{2+} - CO_2 -activated Rubisco activities for plants grown in ambient CO_2 (O) or $900 \mu\text{l CO}_2/\text{liter}$ (●) during the development of flowering shoots for weeks 16 to 20 (Expt. 1). Each point represents four leaves. The vertical lines indicate the standard error of the means.

tosynthesis previously reported by Bozarth et al. (1982) due to decreasing irradiance and increasing leaf age. However, Mg^{2+} - CO_2 -activated activities did not decline with increasing canopy depth. This result indicates the potential for higher photosynthetic rates does not decline with leaf ages up to 7 weeks and suggests no decline in the quantity of activatable Rubisco protein during this relatively short period of shoot development. CA declined with increasing canopy depth. This decline suggests that increases in mesophyll resistance and possibly the lower photosynthetic rates (Bozarth et al., 1982) found in these lower leaves may be associated with slower CO_2 diffusion to the chloroplast due to lower CA activity, but this point warrants further investigation. Higher initial activities for the CO_2 -enriched plants after 20 weeks (mid-May) may indicate light limitations during late winter and early spring when sun angles were low. Balazsovits et al. (1989) reported a positive linear effect of supplemental irradiation on cut-flower yield during the winter and early spring. Canopy densities in May appeared to

Fig. 5. Changes in the (A, B) initial and (C, D) Mg^{2+} - CO_2 -activated Rubisco activities and (E, F) CA during shoot development for plants grown in ambient CO_2 (O) or 900 μL CO_2 /liter (●) (Expt. 2). Activities measured during weeks 2 to 5 are shown in A, C, and E, while those from weeks 6 to 9 are shown in B, D, and F. Each point represents four leaves. The vertical lines indicate the standard error of the means.

From these results, we conclude that there is no consistent difference in either initial or Mg^{2+} - CO_2 -activated Rubisco or CA activities between the plants grown in ambient or CO_2 -enriched atmospheres. Neither the canopy position nor developmental studies provide invariable evidence for superior activity in either CO_2 treatment. This result agrees with previous studies on citrus (Koch et al., 1986), but contrasts with studies on nonwoody species. Maintenance of higher levels of carbon assimilation in woody species may be associated with sustained levels of the Rubisco enzyme. The results presented here lend support to the hypothesis proposed by Yelle et al. (1989) that states the reductions in photosynthesis and productivity of tomatoes grown under CO_2 -enriched atmospheres are related to the lower quantities of the Rubisco enzyme in leaves of these plants than in those of ambient-grown plants.

The sustained higher yields reported for roses under prolonged CO_2 enrichment (Balazsovits et al., 1989; Hanan, 1973; Hand and Cockshull, 1975) are hypothesized to be related to the ability of rose leaves to maintain constantly high levels of

carbon assimilation. Continuous removal of the flowering shoots promotes renewal of the canopy. Developing shoots also place a high demand on leaves for photosynthates (Hanson et al., 1988; Mor and Halevy, 1979; Tschaplinski and Blake, 1989a, 1989b). High sink demand has been shown to maintain higher photosynthetic rates during periods of CO_2 enrichment for nonwoody plants (Mauney et al., 1978; Peet et al., 1986). During production, a single rose plant may have two to seven developing shoots at one time. We suggest that constant removal of flowering shoots during the fall to spring production period, as well as low irradiances, maintains rose plants in a carbohydrate-poor state. This state provides a continuous demand for the extra photosynthates produced with CO_2 enrichment, preventing feedback inhibition that would decrease the quantity of Rubisco and, therefore, photosynthesis.

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