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ORIGINAL ARTICLE

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Photosynthetic characteristics and growth responses of dwarf apple (*Malus domestic*a Borkh. cv. Fuji) saplings after 3 years of exposure to elevated atmospheric carbon dioxide concentration and temperature

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Abstract Growth and photosynthetic responses of dwarf apple saplings (*Malus domestic*a Borkh. cv. Fuji) acclimated to 3 years of exposure to contrasting atmospheric CO_2 concentrations (360 and 650 µmol mol^{-1}) in combination with current ambient or elevated (ambient +5°C) temperature patterns were determined. Four 1-year-old apple saplings grafted onto M.9 rootstocks were each enclosed in late fall 1997 in a controlled environment unit in nutrient-optimal soil. Soil moisture regimes were automatically controlled by drip irrigation scheduled at 50 kPa of soil moisture tension. For the elevated CO_2 concentration alone, overall tree growth was suppressed. However, tree growth was slightly enhanced when warmer temperatures were combined with the elevated CO_2 concentration. Neither temperature nor CO_2 concentration affected leaf chlorophyll content and stomatal density. The elevated CO_2 concentration decreased mean leaf area, but increased starch accumulation, thus resulting in a higher specific dry mass of leaves. An elevated temperature reduced starch accumulation. Light-saturated rates of leaf photosynthesis were suppressed due to the elevated CO_2 concentration, but this effect was removed or enhanced with warmer temperatures. The elevated CO_2 concentration increased the optimum temperature for photosynthesis by ca. 4°C, while the warmer temperature did not. The results of this study suggested that the long-term adaptation of apple saplings to growth at an elevated CO_2 concentration may be associated with a potential for increased growth and productivity, if a doubling of the CO_2 concentration also leads to elevated temperatures.

Keywords Apple · Elevated carbon dioxide concentration · Temperature · Photosynthesis · Growth

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Introduction

Evidence suggests that higher plants respond to a rising ambient atmospheric CO_2 concentration by increasing their CO_2 uptake (Ciais et al. 1995). According to global climate change scenarios, atmospheric CO_2 concentrations are predicted almost double within the twenty-first century if current emissions are not reduced, and this doubling of the CO_2 concentration will increase the mean surface temperature of the earth by about 2–6°C (Burroughs 2001). The atmospheric CO_2 concentration and temperature are concomitant factors influencing the global environment (Morison and Lawlor 1999), so the response of higher plants to rising CO_2 concentration, temperature, and their possible interactions is of significantinterest for future agricultural and natural productivity (Fritschi et al. 1999). Recent studies confirm that the impact of global warming beyond a certain limit may have serious consequences for agricultural productivity (Lal etal. 1998).

One of the more sensitive and intriguing responses of plants to elevated CO_2 and temperature is the acclimation of photosynthesis (Stitt 1991). There is abundant evidence that photosynthesis acclimates to elevated CO_2 (Curtis 1996; Weber et al. 1994). Short-term exposure of plants to elevated CO_2 stimulates the rate of photosynthesis and biomass production (DeLucia et al. 1999). However, the effects of long-term exposure among dif-

ferent plant species are conflicting. In general, prolonged exposure to elevated CO_2 reduces the initial stimulation of photosynthesis in many species, and frequently suppresses photosynthesis, due in part to excess accumulation of starch in leaves, which probably hinders CO_2 diffusion within the chloroplast (Makino 1994; Nafziger and Koller 1976). However, such photosynthetic suppression cannot be so great for species which have strong sink organs for carbohydrate accumulation (Makino and Mae 1999).

In general, an increase in temperature counters the suppression of photosynthesis due to an elevated CO_2 concentration (Drake et al. 1997), but the effects on plant growth are either positive or negative depending on the species (Reddy et al. 1998). From this interaction, it is deduced that the optimum temperature for the maximal rate of CO_2 assimilation must increase by about 6°C with an increase in the CO_2 concentration to 670 µmol mol^{-1} (Long 1991). This interaction between CO_2 and temperature could, therefore, be of profound importance for future agricultural productivity, but there is little information regarding the nature of this interaction (Morison and Lawlor 1999).

The effect of an increased CO_2 concentration on plant growth is primarily due to changes in the composition and dry mass per unit area of leaves (Roderick et al. 1999), and the factors influencing these changes are primarily temperature dependent. Stomatal density is indicative of the extent of the acclimation of photosynthesis to a changing CO_2 concentration (Sage 1994), and generally decreases with an increase in CO_2. However, this decrease is not universally observed, and varies among species (Estiarte et al. 1994; Woodward et al. 1991). In contrast to the general response to an elevated CO_2 concentration, Maherali and DeLucia (2000) observed an increased leaf-specific hydraulic conductivity of Ponderosa pine exposed to elevated temperature. They hypothesized that this response should increase stomatal conductance and, therefore, transpirational cooling.

Apple, one of the commercially important temperate fruits, has been widely cultivated from prehistoric times. The global demand for apples and their products has not slowed down, and thus apple producers face the challenge of producing more apples from less area in an energy-efficient way (Ro and Park 2000). However, it is not clear whether such an increase in productivity can be sustained or achieved if global warming occurs. As elevated atmospheric temperature and CO_2 concentration are expected to be part of our future climate, it is important to understand and quantify the responses of apple trees to these two interactive environmental factors.

We measured the growth responses of dwarf apple (*Malus domesti*ca Borkh. cv. Fuji) saplings after 3 years of exposure to elevated CO_2,to elevated temperature, and to these factors in combination. To understand the mechanisms of the responses, leaf photosynthesis was measured. We hypothesized that: (1) high temperatures would ameliorate the effects of elevated atmospheric CO_2; (2) fruit yield would interact with the effects of atmospheric CO_2, temperature, and their interactions; and (3) long-term exposure to elevated CO_2 would shift the optimum temperature for photosynthesis to a higher temperature.

Materials and methods

CO_2- and temperature-controlled closed environment facility for plant growth

The closed-environment plant-growth facility, sunk into the soil, consists of two rows of four experimental units each. Each unit comprises a soil compartment (3×3×3 m), a transparent canopy enclosure (4×3×6 m), and a utility space for the temperature-control unit located on the north side. A weather station measures air temperature and relative humidity, wind speed and direction, solar radiation, and rainfall using a datalogger (21X, Campbell Scientific, USA). An open-architecture, distributed control system (DCS), POREX 6800 (POSCON Institute 1996), developed by POSCON of POHANG Steel, Korea, was used to control the facility and the automated collection of the sensors' data. POREX 6800 DCS consists of two UNIX-based workstations (SPARCstation 20, Sun Microsystems, USA) providing user-friendly man-machine interfaces for process operation and engineering works, and a process control station performing various real-time processing for field input/output points, and scheduled transfer of real-time data to a central database.

Each unit individually controls atmospheric CO_2 and air temperature. Compressed CO_2 gas is mixed with the flow of fresh air depending on the preset CO_2 concentration of the bulk air in the enclosure, and the mixture is brought into the enclosure by the air blower. The purity of the CO_2 gas was regularly inspected. Chilled or heated water is supplied to the fan coil unit depending on whether cooling or heating is required. Conditioned air passes through the plant canopy with sufficient flux to cause slight leaf flutter, and returns to the outlet duct just above the soil level.

An atmospheric CO_2 concentration at ±1 Pa of a predetermined set value and temperature at ±0.5°C of an ambient regime were individually controlled in each unit. Reference values for the ambient regime were taken real-time from a weather station. The solar radiation, air temperatures and relative humidity inside the enclosure were measured and multiplexed to a 21X datalogger. Atmospheric CO_2 concentrations in the enclosure are measured using infrared CO_2 analysers (ZRH, Fuji Electric, Japan), and the addition of CO_2 prior to inlet points is thus controlled. Elevated temperatures included a 5°C step above the ambient regime. Four profiles of time-domain reflectometry probes were installed horizontally with respect to roots at 0.15-m intervals, 0.15–1.20 m below the soil surface, and three tensiometers at depths of 0.15, 0.45, and 0.75 m were also installed. Soil temperatures were measured from calibrated RTD temperature sensors.

Tree culture under controlled climate conditions

Apple (*M. domestica*) cv. Fuji was selected because itrepresents a large portion of commercial apple production in Korea. Each soil compartment was back-filled with a sandy loam soil (Ro and Park 2000) in 1996, and was stabilized for 2 years. Four nursery apple trees each grafted onto a M.9 rootstock were transplanted in each compartment during late fall in 1997. Four units were maintained at 360 µmol CO_2 mol^{-1}, while the other four were maintained at 650 µmol CO_2 mol^{-1}. The air temperature inside two of the units maintained at 360 µmol CO_2 mol^{-1} mimicked the ambient temperature pattern, while that of the remaining two was kept at an elevated temperature of +5°C. The same temperature treatments were used for the four elevated-CO_2 units. Both CO_2 and temperature treatments were initiated after transplanting and lasted three con-

secutive growing seasons. The relative humidity inside the unit varied mostly between 60% and 80%. Soil moisture regimes were automatically controlled by drip irrigation scheduled at 50 kPa of soil moisture tension, which is the current cultural practice for drip-irrigated apples (Ro and Park 2000). During measurements, the leaf water potential of the trees ranged between –0.1 and –0.4 MPa. Currently recommended N-P-K fertilization rates for apple trees were chosen to maintain optimum foliar nutrient concentrations.

Photosynthetic and growth responses, and leaf morphology

Three fully expanded, mature source leaves from three shoots (leaf number 12 counted from each shoot apex) of each tree were tagged for gas exchange measurements. Gas exchange measurements were done simultaneously with four portable photosynthesis measurement systems (LI-6400, Li-Cor, USA) during a 2-h period, 0900–1100 hours, for several days during the fruit-maturation stage. The light responses of leaf photosynthesis were obtained under two CO_2 partial pressures of 36 Pa and 65 Pa by varying the irradiance inside the chamber of each portable photosynthesis measurement system. Responses were approximated by fitting Michaelis-Menten kinetics to the measured CO_2 fixation rate versus photosynthetic photon flux density (PPFD). Irradiance was chosen in ten steps from 2,000 to 0 µmol m^{-2} s^{-1} of PPFD on the adaxial surfaces of leaves. The CO_2 responses of leaf photosynthesis (A/C_i curves) were obtained at low (150 µmol m^{-2} s^{-1}) and high PPFD (1,500 µmol m^{-2} s^{-1}) with the CO_2 injector system (LI-6400-01, Li-Cor) and liquid CO_2 by varying the CO_2 concentration in the chamber (C_a) from 1,000 to 0 µmol mol^{-1}. The intercellular CO_2 concentration (C_i) was calculated based on the equation of Farquhar and Sharkey (1982). The CO_2 fixation response was also measured by varying leaf temperature under a fixed CO_2 concentration and a PPFD level greater than the light saturation point.

At the end of the experiment, tree growth was assessed using four trees (two treesper unit) per treatment. For each, tree height, crown diameter, shoot length, and numbers of shoots and leaves were measured. Shoot length was determined as the cumulative length of all shoots. A sub-sample of 30 leaves $tree^{-1}$, taken from the middle, was measured with an automatic leaf area meter (LI-3100, Li-Cor), and dry mass of those leaves was determined to calculate specific leaf mass (leaf dry mass per unit leaf area).

Three fully mature source leaves per tree were sampled near tagged leaves for gas-exchange measurements. Chlorophyll was extracted with 10 ml dimethyl sulphoxide solution (Hiscox and Israelstam 1979). The total chlorophyll content of leaves was determined by adding chlorophyll a and b contents determined spectroscopically (MacKinney 1941).Sixteen fully mature source leaves from four shoots (leaf number 13–16 counted from each shoot apex) per tree were sampled before dawn (0500–0530 hours) on 5 September during the fruit maturation stage. The collected leaf samples were immediately oven-dried at 105°C for 30 min, and subsequently at 70°C overnight. Dried leaf samples were ground to pass through a 40-mesh sieve. The starch concentration in leaves was determined by measuring the glucose concentration of amyloglucosidase digests following a modified procedure of Reddy et al. (1998) with a HPLC (SP8800, Spectra-Physics, USA) equipped with a SugarPak-1 column and a RISE-61 refractive index detector. Stomatal density was counted on scanning electron photomicrographs (200×) taken with a S-2460 N SEM (Hitachi, Japan) on the same portion of abaxial surfaces of three randomly selected leaves per tree. Ultra-thin sections obtained from leaf transverse sections taken at the point of maximum leaf width across the main vein were stained and examined with a transmission electron microscope (LEO-906E, Zeiss, Germany).

Statistical analysis

Data were evaluated using General Linear Models procedures (SAS Institute 1990). Data were analysed with Tukey's studentized range (honestly significant difference) test after a two-way ANOVA for the completely randomized design to compare the significance of two factors and the effects of temperature and CO_2 concentration treatments as well as the effects of their interaction at the significance level of 0.05. Additionally, least square difference was applied to test the significance among treatments. Finally, data were tested by multivariate ANOVA (MANOVA) to examine response variables.

Results and discussion

Tree growth

The elevated temperature significantly increased tree height, crown diameter, shoot length, and numbers of shoots and leaves, while elevated CO_2 did not (Table 1). There was a significant temperature×CO_2 interaction for tree height, shoot length, and numbers of shoots and leaves, but not for crown diameter. Despite the insignificant effect with regard to crown diameter, tree growth was enhanced under elevated CO_2 when coupled with elevated temperature, but was suppressed under elevated CO_2 alone, compared to trees grown under ambient temperature and CO_2 concentration (control). The decreased tree growth after long-term exposure to elevated CO_2 was similar to results with other C_3 plants, where bio-

Table 1 Effects of atmospheric temperature and CO_2 concentration on crown diameter, tree height, shoot length, and numbers of shoots and leaves. $LSD_{0.05}$ least significant difference at 0.05 level

Temperature	CO_2 concentration (µmol mol^{-1})	Crown diameter (cm)	Tree height (cm)	Shoot length (cm)	Number of shoots	Number of leaves
Ambient	360	210.8	268.2	1,793.3	38.8	1,313.8
	650	187.4	273.4	1,299.8	34.0	864.5
Ambient+5°C	360	224.8	276.4	1,667.7	43.3	1,082.0
	650	236.0	332.0	1,961.7	50.3	1,383.5
$LSD_{0.05}$		40.1	27.3	176.1	7.0	189.8
ANOVA						
Temperature		*	***	***	***	*
CO_2 concentration		n.s.	n.s.	n.s.	n.s.	n.s.
Temperature×CO_2 concentration		n.s.	***	***	*	***

***P<0.001, **P<0.01, *P<0.05, *n.s.* not significant

mass production was reduced due to a decreased rate of photosynthesis (Drake et al. 1997).

In contrast, simultaneous exposure to increased temperature and CO_2 resulted in a significant increase in height, diameter, and dry mass of seedlings in another study (Sheu and Lin 1999). Farrar and Williams (1991) suggested that higher temperatures would increase the degree of inorganic phosphate recycling to the chloroplast bystimulating sucrose synthesis and hence permit enhanced rates of assimilate production for CO_2-enriched leaves. In addition, temperature increased the transport of assimilates and sink metabolism, and this might explain increases in tree growth above that seen for CO_2-enriched trees alone.

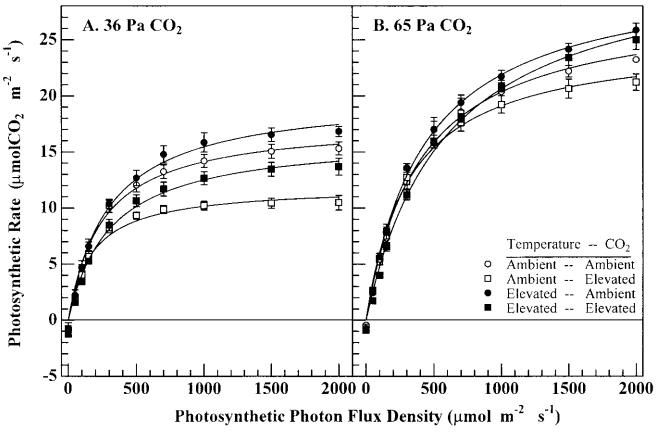

Fig. 1 Light-response curves for photosynthesis of leaves measured at 36 Pa (**A**) and 65 Pa (**B**) partial CO_2 pressure. *Vertical bar* at each data point denotes ±SD of the mean when *larger* than symbol size

Light-response curves of photosynthesis

Leaves grown in elevated CO_2 demonstrated a lower rate of photosynthesis than leaves grown in ambient CO_2. When measured at PPFD levels greater than the light saturation point under 36 Pa CO_2 partial pressure (Fig. 1A), the photosynthetic mechanism of leaves of trees was acclimated to elevated CO_2 and, as a result, leaves had lower maximum net photosynthesis. Pan et al. (1998) observed a suppression of photosynthesis in 1-year-old maiden apple (cv. Gala) plants following 8 days of exposure to elevated CO_2, but the values were still higher than control values. In both their and our studies, when light intensity was inadequate, the light-harvesting abilities of leaves exposed to elevated CO_2 were also lower than those exposed to ambient CO_2. In general, suppression of photosynthesis of plants grown in elevated CO_2 relative to control plants is apparent when both are measured at ambient CO_2 levels (Drake et al. 1997). Elevated temperature, however, increased the rate of photosynthesis of leaves exposed to ambient or elevated CO_2 concentrations. This implies that the stimulatory effects of elevated temperature offsets the reduction in photosynthesis due to elevated CO_2 (Farrar and Williams 1991).

On the other hand, the rate of photosynthesis measured at 65 Pa CO_2 was higher than that measured at ambient CO_2 due to the increase in the CO_2 concentration (Fig. 1B). In particular, a 1-h exposure to elevated CO_2 during measurements stimulated the rate of photosynthesis of trees grown at ambient CO_2 concentration, thus suggesting that the current ambient CO_2 concentration is insufficient to saturate Rubisco (Drake et al. 1997). As mentioned, long-term acclimation to elevated CO_2 suppressed photosynthesis unless accompanied by exposure to warmer temperatures. Nagy et al. (2000) observed that the maximum net photosynthesis of elevated-CO_2 trees increased more during warmer seasons than during cooler seasons under their experimental conditions. It was also noted that the light-saturated rate of photosynthesis seemed to increase even at 2,000 μmol m^{-2} s^{-1} under this elevated CO_2 partial pressure (65 Pa).

Compared to light-saturated responses under 36 Pa CO_2 (Fig. 1A), the increasing effect due to elevated temperature was similar irrespective of treatment CO_2 levels. In particular, regardless of growth temperature, a sudden exposure of elevated-CO_2 grown leaves to 36 Pa CO_2 immediately increased the difference (2.0–5.4 μmol m^{-2} s^{-1} for ambient, and 0.9–3.2 μmol m^{-2} s^{-1} for elevated temperature conditions) in the light-saturated rate of photosynthesis between the two CO_2 treatments, suggesting a probable impedance to intracellular CO_2 diffusion (Makino 1994).

Light saturation and compensation points, dark respiration, maximum net photosynthesis, and apparent quantum yield

The light saturation point was greater under exposure to 65 Pa CO_2 (816 μmol m^{-2} s^{-1} for control trees) than under exposure to 36 Pa CO_2 (622 μmol m^{-2} s^{-1} for control trees), while the reverse (14 μmol m^{-2} s^{-1} at 36 Pa and 10 μmol m^{-2} s^{-1} at 65 Pa CO_2 for control trees) was the case for the light compensation point (Table 2).

An elevated temperature and CO_2 concentration significantly increased dark respiration. Several reports have found a decrease in dark respiration during sudden exposure to elevated CO_2 (Amthor et al. 1992). However, the dark respiration of *Chamaecyparis obtusa* increased with an increasing CO_2 concentration and temperature (Nagy et al. 2000). Maximum net photosynthesis (A_{max}) ranged from 9.9 to 25.9 μmol m^{-2} s^{-1}. For trees grown at a given CO_2 level, increasing the measurement CO_2 level increased A_{max} (e.g. from 15.3 to 23.2 and from 9.9 to 21.2 μmol m^{-2} s^{-1}). On the other hand, for a given measurement CO_2 level, increasing the treatment CO_2 level decreased A_{max} (from 15.3 to 9.9 and from 23.2 to 21.2 μmol m^{-2} s^{-1}). Long-term exposure to an elevated temperature did not greatly change the pattern described above, but significantly reduced the degree of suppression of A_{max} due to elevated CO_2. Overall, temperature did have an appreciable effect on the light com-

Table 2 Effects of atmospheric temperature and CO_2 concentration on light saturation and compensation points, dark respiration, maximum net photosynthesis, and apparent quantum yield of leaves measured at 36 and 65 Pa CO_2 partial pressure. $LSD_{0.05}$ least significant difference at 0.05 level

Temperature	CO_2 concentration (μmol mol^{-1})	Light compensation point (μmol m^{-2} s^{-1})		Light saturation point (μmol m^{-2} s^{-1})		Dark respiration (μmol CO_2 m^{-2} s^{-1})		Maximum net photosynthesis (μmol CO_2 m^{-2} s^{-1})		Apparent quantum yield (mmol CO_2 mol^{-1})	
		36 Pa	65 Pa	36 Pa	65 Pa	36 Pa	65 Pa	36 Pa	65 Pa	36 Pa	65 Pa
Ambient	360	14	10	622	816	0.8	0.5	15.3	23.2	35.5	41.8
	650	18	9	371	652	0.8	0.6	9.9	21.2	26.3	44.3
Ambient	360	14	11	682	968	0.8	0.8	16.8	25.9	36.0	46.8
+5°C	650	25	18	695	1,019	1.3	0.9	13.7	25.0	31.0	39.8
$LSD_{0.05}$		2	2	38	121	0.2	0.1	1.4	1.2	2.9	3.3
ANOVA											
Temperature		***	***	***	***	**	***	***	***	*	n.s.
CO_2 concentration		***	**	***	n.s.	***	***	***	**	***	n.s.
Temperature×CO_2 concentration		***	***	***	*	**	n.s.	*	n.s.	*	***

***P<0.001, **P<0.01, *P<0.05, *n.s.* not significant

Table 3 Effects of atmospheric temperature and CO_2 concentration on chlorophyll and starch contents, stomatal density, mean area, and specific dry mass of leaves. $LSD_{0.05}$ least significant difference at 0.05 level

Temperature	CO_2 concentration (μmol mol^{-1})	Chlorophyll content (g m^{-2})	Starch content (g kg^{-1})	Stomatal density (no mm^{-2})	Mean leaf area (cm^2 leaf^{-1})	Specific leaf dry mass (mg cm^{-2})
Ambient	360	2.07	7.73	340.3	42.0	8.2
	650	2.07	16.80	285.4	29.8	9.9
Ambient+5°C	360	2.18	8.60	301.1	42.0	9.1
	650	2.10	10.90	339.8	38.9	10.1
$LSD_{0.05}$		0.34	1.28	42.0	6.9	0.9
ANOVA						
Temperature		n.s.	***	n.s.	n.s.	n.s.
CO_2 concentration		n.s.	***	n.s.	**	***
Temperature×CO_2 concentration		n.s.	***	**	n.s.	n.s.

***P<0.001, **P<0.01, *n.s.* not significant

pensation and saturation points, dark respiration, and A_{max}.

Apparent quantum yield (Table 2) was estimated by fitting a linear regression to the measured rate of photosynthesis versus PPFD over the range 0–300 μ mol m^{-2} s^{-1} (Evans 1987). Elevated CO_2 significantly suppressed the apparent quantum yield at 36 Pa CO_2, but the degree of suppression was significantly reduced by elevated temperature. However, temperature and CO_2 concentration did not affect the apparent quantum yield at 65 Pa CO_2, indicating a significant interaction between temperature and CO_2 concentration. Overall, the apparent quantum yield was greatest in trees grown at elevated CO_2 with a concurrent increase in temperature.

Leaf characteristics

Neither temperature nor CO_2 concentration significantly affected chlorophyll content and stomatal density of leaves (Table 3). However, there was a significant temperature×CO_2 concentration interaction for stomatal density. Woodward (1987) compared stomatal density in herbarium material from the mid-nineteenth century to the present day and observed declines, but several studies found that atmospheric CO_2 concentrations did not affect stomatal density (Estiarte et al. 1994). Elevated CO_2 significantly decreased the mean area of individual leaves (Sheu and Lin 1999), but increased specific leaf dry mass (Sage et al. 1989). Pan et al. (1998) observed an increase in specific dry mass of apple leaves, and suggested that it was due to the accumulation of starch. However, temperature did not significantly affect the mean area and specific dry mass of leaves.

Starch accumulation

Elevated CO_2 led to a significantly greater accumulation of starch in leaves. In contrast, elevated temperature led to a decrease in starch (Table 3). Transmission electron photomicrographs of mesophyll cells showed more starch grains in the leaves grown in elevated CO_2, and reduced starch accumulation with concurrent exposure to elevated temperature (Fig. 2). Elevated CO_2 usually led to an increased rate of photosynthesis in plants when measured at

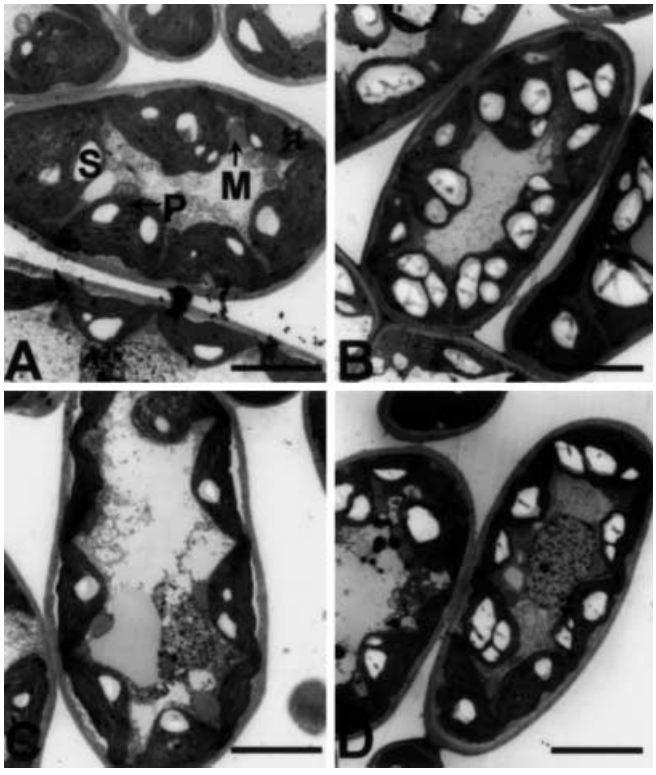

Fig. 2 Transmission electron photomicrographs of mesophyll cells showing starch grains (*S*), and arrangement of mitochondria (*M*) and plastoglobuli (*P*) within a cell. Ambient (*A*) or elevated (*B*) CO_2 concentration with ambient temperature pattern, and ambient (*C*) or elevated (*D*) CO_2 concentration with a temperature pattern 5°C above ambient. *Scale bar*=5 µm

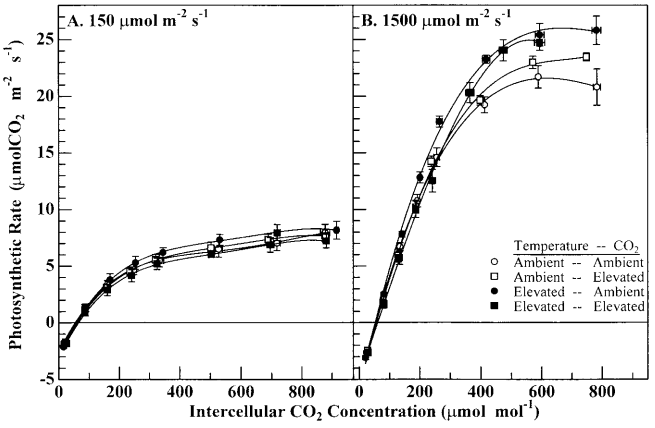

Fig. 3 CO_2 response curves for photosynthesis of leaves measured at 150 µmol m^{-2} s^{-1} (**A**) and 1,500 µmol m^{-2} s^{-1} (**B**) photosynthetic photon flux density. *Vertical bar* at each data point denotes ±SD of the mean when *larger* than symbol size

the same CO_2 concentration at which they were grown (Fig. 1). In addition, Stitt (1991) noted increased starch accumulation. In contrast, an elevated temperature decreased starch accumulation in elevated-CO_2 grown leaves (Farrar and Williams 1991). Starch accumulation may increase the resistance to intracellular CO_2 diffusion in elevated-CO_2 grown leaves or lead to a down-regulation ofRubisco; however, the presence of a causal relationship between starch accumulation and the inhibition of photosynthesis remains controversial (Stitt 1991).

The accumulation of starch within leaves of CO_2-enriched plants may reflect a temporary or permanent imbalance between sources and sinks. If sink capacity is low, an accumulation of photosynthate may occur within source leaves leading to feedback inhibition of photosynthesis; otherwise photosynthesis is stimulated (Herold 1980). Reduced overall tree growth and significant accumulation of starch in leaves were observed in elevated-CO_2 grown trees. In contrast, for trees grown under both elevated CO_2 and temperature, starch accumulation was reduced, due probably to the increased utilization of stored assimilates for the growth of sink organs within a whole tree (Tables 1, 3). For instance, fruit yield per tree averaged for 2 years was 1.4 kg for trees grown in an elevated CO_2 concentration alone, but increased to 4.3 kg with a higher temperature (unpublished data).

CO_2 response curves of photosynthesis

When the light intensity was limiting (i.e. 150 µmol m^{-2} s^{-1}), there was no treatment-related difference in A/C_i curves (Fig. 3A). Analyses of these A/C_i curves showed that the initial slope was as steep as that in Fig. 3B, and the C_i value at which CO_2 saturation occurred was not greatly altered between treatments, thus indicating that the balance between Rubisco and RubP regeneration was being maintained (Evans 1988). CO_2 saturation occurred at a C_i value close to that found at ambient (36 Pa) CO_2 partial pressure (Fig. 1A).

When light intensity was not limiting (Fig. 3B), leaves grown at ambient CO_2 and elevated temperature had slightly higher rates of photosynthesis compared to when light was limiting. However, when photosynthesis was CO_2 saturated, the photosynthetic capacity increased with elevated temperature, regardless of the growth CO_2 conditions. Compared to control trees, despite the insignificance of the initial slope, elevated-CO_2 grown trees had a slightly lower initial slope and a less sensitive response when photosynthesis was measured at low C_i than at high C_i values. The decrease in the initial slope of the A/C_i response suggested that the amount of Rubisco was lower in elevated-CO_2 grown trees (Stitt 1991).

After the CO_2 saturation point, the rate of photosynthesis of leaves exposed to elevated temperature and ambient CO_2 was significantly higher. In general, trees grown at elevated temperature had a higher rate of photosynthesis than trees grown at ambient temperature, regardless of the CO_2 concentration in which they were grown. Although at a given CO_2 partial pressure (36 Pa), the elevated-CO_2 grown trees had about two-thirds the rate of photosynthesis of their counterparts grown at ambient CO_2 (Fig. 1A), they had similar intercellular CO_2 concentrations (about 260 µmol mol^{-1}). This suggested that the lower supply of CO_2 to the mesophyll cells, as a result of increased stomatal resistance, was counterbalanced by a decreased utilization of CO_2 molecules

Table 4 Effects of atmospheric temperature and CO_2 concentration on CO_2 compensation point, carboxylation efficiency, and photorespiration of leaves measured at photosynthetic photon flux density levels of 150 and 1,500 µmol m^{-2} s^{-1}. $LSD_{0.05}$ least significant difference at 0.05 level

Temperature	CO_2 concentration (µmol mol^{-1})	Photo-respiration (µmol CO_2 m^{-2} s^{-1})		Carboxylation efficiency (µmol CO_2 m^{-2} s^{-1})		CO_2 compensation point (µmol CO_2 mol^{-1})	
		150 µmol m^{-2} s^{-1}	1,500 µmol m^{-2} s^{-1}	150 µmol m^{-2} s^{-1}	1,500 µmol m^{-2} s^{-1}	150 µmol m^{-2} s^{-1}	1,500 µmol m^{-2} s^{-1}
Ambient	360	1.64	4.41	0.028	0.080	59	55
	650	1.88	4.44	0.029	0.081	65	57
Ambient+5°C	360	2.01	4.58	0.031	0.088	65	54
	650	2.07	4.73	0.029	0.077	65	61
$LSD_{0.05}$		0.19	0.46	0.003	0.006	12	4
ANOVA							
Temperature		***	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2 concentration		*	n.s.	n.s.	*	n.s.	**
Temperature×CO_2 concentration		n.s.	n.s.	n.s.	**	n.s.	*

***P<0.001, **P<0.01, *P<0.05, *n.s.* not significant

(Spencer and Bowes 1986). When C_i reached 600 µmol mol^{-1}, the rate of photosynthesis in control trees declined, implying an inhibition to the RubP-regenerating mechanism (Stitt 1991).

Neither temperature nor CO_2 concentration affected carboxylation efficiency and the CO_2 compensation point measured at 150 µmol m^{-2} s^{-1}, and photorespiration measured at 1500 µmol m^{-2} s^{-1} (Table 4). Instead, they significantly increased photorespiration at 150 µmol m^{-2} s^{-1}. However, carboxylation efficiency and the CO_2 compensation point at 1,500 µmol m^{-2} s^{-1} were significantly affected by CO_2 concentration, but not by temperature.

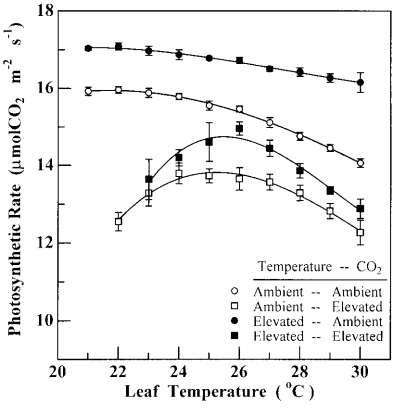

Fig. 4 Temperature-response curves for photosynthesis of leaves. *Vertical bar* at each data point denotes ±SD of the mean when *larger* than symbol size

Temperature-response curves of photosynthesis

The elevated CO_2 concentration suppressed the rate of CO_2 fixation and increased the optimum temperature for maximal CO_2 uptake of leaves of trees by 4°C, regardless ofwhether they were grown at ambient or elevated temperature conditions (Fig. 4). These findings corroborate the prediction of Long (1991) that the temperature optimum for photosynthesis must increase with CO_2 concentration. However, an elevated temperature did not change the optimum temperature for maximal CO_2 uptake. Leaves of trees grown at an elevated temperature had a significantly higher CO_2 fixation rate than the leaves of trees grown at ambient temperature conditions, regardless of the CO_2 concentration to which they were exposed. This indicates that the CO_2 concentration interacted with temperature, and that the optimum temperature for photosynthesis increased with increasing CO_2 concentration. When elevated CO_2 led to an accumulation of starch, an increased temperature caused starch to decrease (Fig. 2, Table 3).

Considerable research has been done on the effects of elevated CO_2 and elevated temperature on plant productivity. However, little is known about the interaction between elevated CO_2 and elevated temperature on plant growth (Nagy et al. 2000). In addition, the responses of the deciduous apple tree to elevated CO_2 and temperature may differ from other C_3 plants, because apple, a Rosaceae, synthesizes and translocates sorbitol, a sugar alcohol, in addition to sucrose (Webb and Burley 1962; Wallart 1980). Our study thus lacks supporting information about the partitioning of assimilates and their translocation between source and sink organs within the whole tree during growth. In particular, carbohydrate reserves in apple trees play an essential role during the early part of the next spring's growth (Hansen 1971). Some studies suggested that higher carbohydrate levels associated with low N availability might result in a large decrease in photosynthesis (Paul and Driscoll 1997). During our study, leaves were not deficient in N: 20.5±1.4 g N kg^{-1} leaf for 3 years. Particularly, trees grown under elevated CO_2 alone had higher leaf N concentrations than the rest: 19.4 g N kg^{-1} leaf for 1998, 21.2 g N kg^{-1} leaf for 1999, and 21.5 g N kg^{-1} leaf for 2000, respectively. However, the leaf N concentration in trees grown at elevated CO_2 and temperature was lower than that of control trees as a result of the dilution of N as a consequence of growth.

Similar to our study, Farrar and Williams (1991) found that the effects of increasing CO_2 and temperature were to some extent opposite. Plants grown in warmer environments have less stored carbohydrates, particularly starch; plants in high-CO_2 environments have more. Consequently, the increased supply of assimilates provided under a high-CO_2 environment and the increased sink strengths permitted by warming should combine to produce larger plants (Table 1) with less inhibition of photosynthesis and a higher flux of carbohydrates whatever their pool size (Fig. 1 and Fig. 2). Our study showed that the rate of photosynthesis did not decrease in high-CO_2 and -temperature grown leaves. However, without a study of the flux of carbohydrate, this study could not clearly explain how a warmer temperature induces an increase in tree growth and productivity and a decrease in starch accumulation in leaves of CO_2-enriched apple trees.

In the long-term, the ability of apple leaves to sustain higher photosynthetic rates would depend on the sink-source status of the whole tree and how this is regulated. Therefore, physiological and biological mechanisms that regulate sink-source interactions need further study.

We concluded that: (1) warmer temperature (5°C above ambient) counteracted the suppression of growth and photosynthesis of apple saplings due to an enhanced CO_2 concentration; (2) fruit yield was reduced by an elevated CO_2 concentration, but enhanced with concurrent warmer temperature, thus in turn affecting starch accumulation in leaves and the growth of whole trees; and (3) 3 years of exposure to elevated CO_2 concentration increased the optimum temperature of photosynthesis by 4°C, while warmer temperature did not. Despite the insignificance of several response variables, MANOVA of all response variables showed that overall responses were significantly influenced by temperature, CO_2 concentration, and their interactions. Part of this study demonstrated that the stimulation of photosynthesis of dwarf apple saplings by an elevated CO_2 concentration did not disappear for 3 years, and suggested that the long-term adaptation of apple saplings to growth at an elevated CO_2 concentration may be associated with a potential for increased growth and productivity, if a doubling of the CO_2 concentration causes a simultaneous increase in the atmospheric temperature.

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