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Résumé de l'article

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Des semis d'érable à sucre de 2 ans ont été exposés en chambre à ciel ouvert pendant 86 jours à trois fois la concentration ambiante de O_3 (3x) et à une forte concentration de CO_2 (650 µL L^{-1}), seul ou en combinaison. Le taux d'assimilation du CO_2, la croissance des semis et les cires cuticulaires n'ont pas été modifiés par le traitement oxydatif après une saison de croissance. L'absence de réponse sous O_3 est attribuée à la faible conductance stomatique de l'érable à sucre et à l'entrée réduite de O_3 dans les feuilles qui en découle. De plus, l'alternance de périodes où les concentrations de O_3 sont faibles et de périodes où les concentrations de O_3 sont élevées a probablement permis aux semis de contrer le stress oxydatif. À la fin du mois d'août, la biomasse et le rapport assimilation nette de CO_2/conductance stomatique au CO_2 mesurés dans les deuxièmes pousses des semis exposés au fort CO_2 ont doublé par rapport aux semis exposés au CO_2 ambiant. Les conditions environnementales à l'intérieur des chambres (bonne disponibilité en lumière, éléments minéraux, eau) ont permis aux semis de profiter de la forte disponibilité en CO_2. Les semis ont un avantage compétitif en termes de croissance dans les conditions environnementales de fort CO_2 alors que trois fois la concentration ambiante de O_3 n'a pas diminué l'effet fertilisant du fort CO_2.

Response of *Acer saccharum* seedlings to elevated O_3 and CO_2 concentrations

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The effects of three times ambient [O_3] (3x) and high [CO_2] (650 μL L^{-1}) alone and in combination were studied on 2-yr-old sugar maple (*Acer saccharum*) seedlings for 86 days in open top chambers. Sugar maple net CO_2 assimilation rate and growth were not decreased by the O_3 treatment after one growing season, and the epicuticular wax was not damaged compared with the control. The absence of response to the O_3 treatment is attributable to the low stomatal conductance of this species resulting in a low O_3 uptake, together with the succession of periods of high and low [O_3], which allowed the seedlings to alleviate the oxidative stress. At the end of August, under high [CO_2], the growth of the seedlings and net CO_2 assimilation to stomatal conductance to CO_2 ratio in the second flush of leaves had doubled. Under the environmental growth conditions of the chambers (high light, nutrients and water availabilities), the seedlings may benefit from the availability of CO_2. Sugar maple seedlings may have a competitive growth advantage under elevated CO_2 conditions and three times ambient [O_3] did not decreased the fertilizing effect of CO_2.

Keywords: CO_2 assimilation, elevated CO_2, elevated O_3, epicuticular wax, growth, sugar maple.

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Des semis d'érable à sucre de 2 ans ont été exposés en chambre à ciel ouvert pendant 86 jours à trois fois la concentration ambiante de O_3 (3x) et à une forte concentration de CO_2 (650 μL L^{-1}), seul ou en combinaison. Le taux d'assimilation du CO_2, la croissance des semis et les cires cuticulaires n'ont pas été modifiés par le traitement oxydatif après une saison de croissance. L'absence de réponse sous O_3 est attribuée à la faible conductance stomatique de l'érable à sucre et à l'entrée réduite de O_3 dans les feuilles qui en découle. De plus, l'alternance de périodes où les concentrations de O_3 sont faibles et de périodes où les concentrations de O_3 sont élevées a probablement permis aux semis de contrer le stress oxydatif. À la fin du mois d'août, la biomasse et le rapport assimilation nette de CO_2/conductance stomatique au CO_2 mesurés dans les deuxièmes pousses des semis exposés au fort CO_2 ont doublé par rapport aux semis exposés au CO_2 ambiant. Les conditions environnementales à l'intérieur des chambres (bonne disponibilité en lumière, éléments minéraux, eau) ont permis aux semis de profiter de la forte disponibilité en CO_2. Les semis ont un avantage compétitif en termes de croissance dans les conditions environnementales de fort CO_2 alors que trois fois la concentration ambiante de O_3 n'a pas diminué l'effet fertilisant du CO_2 élevé.

Mots clés : Assimilation de CO_2, cires cuticulaires, concentrations élevées de CO_2, concentrations élevées de O_3, croissance, érable à sucre.

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INTRODUCTION

It is well established that the CO_2 level is a major contributory factor in the global climate change. It has increased in the atmosphere from 280 ppm in 1880 to 365 ppm in 2000, and will double by 2100 (Conway *et al.* 1988). Its effect on the increase of biomass has been widely studied (Drake *et al.* 1997; Saxe *et al.* 1998). Photosynthesis of C_3 plants may increase by 25 to 75% in response to doubling [CO_2] (Kirschbaum 2000). Even at this high CO_2 level and under the anticipated increased temperature (increase of more than 3°C during this century, Saxe *et al.* 2001), the photosynthetic capacity will not be saturated with CO_2 and will further increase with increasing [CO_2] (Kirschbaum 2000). However, many studies have measured a photosynthetic down-regulation after long-term exposure to high [CO_2], especially when water and nutrient supplies are not adequate or when the strength of the sink tissue decreases (Broadmeadow and Jackson 2000; Ceulemans and Mousseau 1994; Crous and Ellsworth 2004; Gunderson and Wullschleger 1994).

Tropospheric [O_3] is increasing in parallel with CO_2, as the precursors of O_3 (NOx, COv) are mainly produced by anthropogenic fossil fuel combustion (Isaksen and Hov 1987). O_3 is one of the most phytotoxic gaseous pollutants that cause oxidative stress (Heath 1980). Following an O_3 exposure, protective properties of the cuticule may be reduced as the degradation of epicuticular waxes may lead to an increased pollutant uptake. Barnes *et al.* (1988) reported changes in surface waxes in response to O_3 similar to those naturally occurring during needle aging. Under O_3 exposure, toxic free radicals are rapidly formed in the leaf tissues. This generally leads to a decrease in the rate of photosynthesis and in biomass accumulation (Gravano *et al.* 2004; Reich *et al.* 1986). The impacts of O_3 depend on the level and duration of external exposure to O_3 (cumulative O_3 dose), the internal O_3 dose received by plants, the mode of fumigation and the level of sensitivity of the species (Bortier *et al.* 2001; Reich 1987).

Sugar maple (*Acer saccharum* Marsh.) is a widely distributed deciduous species in North America with a major economical interest. Sap production generates $140 million per year and sugar maple is a high quality timber. In Quebec, sugar maple seedlings are used in plantations. Thus, seedlings in sugar maple stands and in plantations will be exposed to increasing CO_2 levels, which may lead to an improved growth (Bazzaz *et al.* 1990). However, increasing O_3 levels may impose more frequent oxidative stress periods, which may counteract this advantage (Rebbeck and Scherzer 2002; Rebbeck *et al.* 2004; Riikonen *et al.* 2005). Sugar maple has been reported as a tolerant species to O_3 (Laurence *et al.* 1996; Rebbeck and Loats 1997). However, other studies have shown that this species can be affected by O_3 (Kress and Skelly 1982; Tjoelker *et al.* 1995). The response of epicuticular waxes of sugar maple to an O_3 exposure has never been reported before and will be evaluated in this study. Knowledge of the growth response of sugar maple seedlings to O_3 in an elevated CO_2 atmosphere may give very pertinent information on the response to pollutants during the seedling stage, when competition and growth rate are very high (Bazzaz 1992), and may be helpful for the management of maple stands and plantations.

In this study, young sugar maple seedlings were exposed to CO_2 and O_3 levels similar to those expected before the end of the century to evaluate the impacts of the two gases on their development. The growth of the seedlings, the net CO_2 assimilation rate as well as N assimilation by the nitrate reductase were measured. The potential deleterious effects of O_3 on leaf epicuticular wax and wettability were evaluated by measuring the epicuticular wax quantity and the contact angle.

Table 1. Monthly O_3 mean, daily seasonal mean, cumulative O_3 dose, air and soil temperature (°C), relative humidity (%) in the field (ambient) and in the open-top chambers (OTC) (mean ± SD) in 1995

		June	July	August	Mean	Cumulative dose
		nL L^{-1}				ppm h
O_3 mean	ambient	41 ± 13	36.4 ± 11	29.8 ± 11	36 ± 12	
	1x	42.2 ± 13	33.9 ± 11	27.9 ± 10	35 ± 12	48.8
	3x	112.7 ± 37	108.1 ± 33	90.3 ± 31	104 ± 35	143.7
Air temperature	ambient	=[a]	20.3 ± 2.7	18.8 ± 3.2		
	OTC	=	21.7 ± 2.9	20.0 ± 3.2		
Soil temperature	ambient	=	22.3 ± 2.6	19.0 ± 2.7		
	OTC	=	21.9 ± 2.2	20.0 ± 2.8		
RH	ambient	34.4 ± 11.3	50.6 ± 14.9	41.2 ± 9.7		

[a] = : not determined.

MATERIAL AND METHODS

Growth of the seedlings and fumigation treatments

On 10 May 1995, 48 2-yr-old nursery-grown, bare-root sugar maple seedlings (*Acer saccharum*) were potted in 16 L pots in a substrate (sandy loam rich in organic matter). The sugar maple seedlings grew for two years in a sandy loam with sufficient water and fertilizers in the Berthierville nursery (Ministère des Ressources naturelles, Québec, Canada). Seedlings were equally distributed in eight open-top chambers and allowed to acclimate for 21 d. The chambers used were similar to those described by Heagle *et al.* (1989) (without a rain cap) and were located at the "Centre de recherche acéricole du MAPAQ" in Tingwick, approximately 200 km east of Montreal (45°54' N and 71°57' W). The following treatments were administered in duplicates from 31 May to 25 August 1995 (86 d; daylight hour basis: 5:00 to 20:00): 1) control = 1x $1CO_2$: ambient O_3 concentration (1x) + 350 μL L^{-1} CO_2; 2) 3x $1CO_2$: 3 times ambient O_3 (3x) + 350 μL L^{-1} CO_2; 3) 1x $2CO_2$: 1x O_3 + 650 μL L^{-1} CO_2 and 4) 3x $2CO_2$: 3x O_3 + 650 μL L^{-1} CO_2. The air entering the chambers was filtered with activated charcoal to remove pollutants prior to ozone enrichment. The ventilation rate was ~ 85 m^3 min^{-1}. Monthly [O_3] means, seasonal [O_3] means, cumulative O_3 dose outside the chambers and inside the 1x and 3x chambers are shown in Table 1. Variations in [O_3] outside and inside the chambers are shown in Figure 1.

[O_3] was measured hourly in the centre of the chamber (at 120 cm). Hourly control and feedback adjustments of the O_3 level were made using two UV-photometric O_3 analyzers (Monitor Labs Inc., model 8810, Englewood, CO, USA) linked to a data logger (Campbell Scientific Canada, model CR10, Edmonton, AB). Ozone was generated from dried ambient air using an OREC autocontrol ozonator (Ozone Research & Equipment Corporation, model 03SP38-ARW, Phoenix, AZ, USA) linked to the data logger for feedback control. Preliminary tests did not show any significant NOx increase in the outcoming air. Pure CO_2 was delivered 24 h a day and concentrations were measured hourly in the centre of the chamber (at 120 cm). [CO_2] may fluctuate by 10%. A more complete description of the chambers can be found in Renaud *et al.* (1997).

Each seedling was watered biweekly with 2 L of tap water. Fertilization (6 g L^{-1} of 10-52-10, 2L per pot) was applied on 18 and 29 May, and then the seedlings received a fertilization of 28-14-14 (2 g L^{-1}, 2L per pot) on 22 June, 29 June and 7 July. Irradiance was measured using a quantum sensor (Li-190SA, Li-COR, Lincoln, NE) at noon during each harvest. At noon on a sunny day, irradiance inside the chambers was 80% (1500 µmol m^{-2} s^{-1}) of the full sunlight intensity measured in the field. The mean daily PPFD was 952 µmol m^{-2} s^{-1} (52% of full sunlight). The air and soil temperatures inside and outside the chambers as well as relative humidity outside the chambers were monitored hourly and are presented in Table 1.

Morphological types of seedlings

Budbreak occurred in mid-May during acclimation. Two seasonal patterns of shoot growth were observed among the seedlings. Part of the seedlings had a "truncated" shoot growth pattern such as described by Canham *et al.* (1999) as a "cessation of aboveground growth early in the growing season". These seedlings with one leaf flush will be referred to as "truncated" seedlings. The other part of the seedlings had an "episodic" growth strategy (Canham *et al.* 1999): a second flush of leaves occurred between the end of June and mid-July.

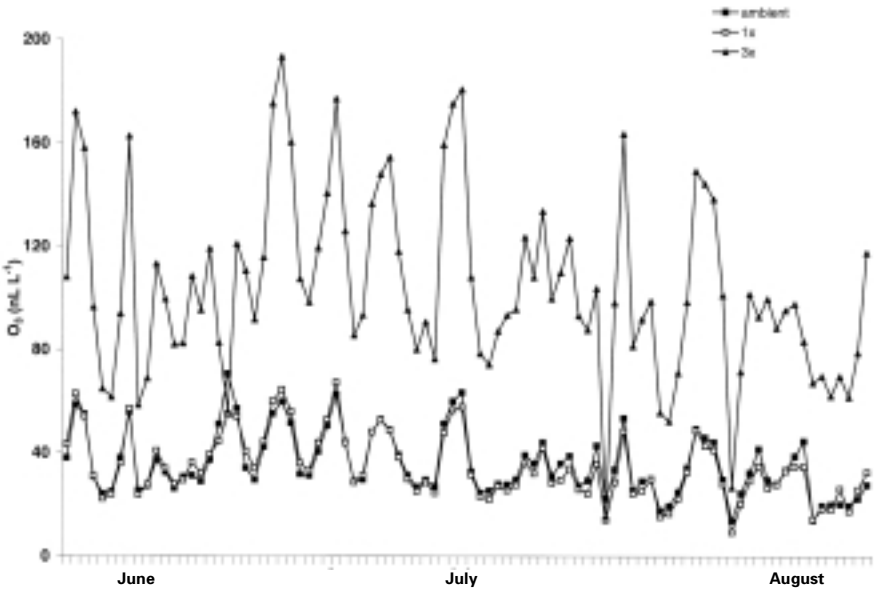

Figure 1. Daily O_3 mean (nL L^{-1}) in the field (ambient), 1x and 3x chambers from May to August 1995.

These seedlings with two flushes will be referred to as "episodic" seedlings. The first flush of leaves will be hereafter referred to as the preformed flush, consisting of preformed leaves, and the second flush as neoformed flush, consisting of neoformed leaves as described by Gregory (1980).

Harvest of the seedlings

Two seedlings per chamber were randomly harvested on 21 June (21 d of treatment), 30 July (60 d of treatment), and 25 August (86 d of treatment). Harvesting started at 13:00 and was completed within 4 h.

At d 21, we collected two truncated seedlings per chamber for a total of four seedlings per treatment. At d 60 and 86, we collected truncated or episodic seedlings for a total of four seedlings per treatment; due to the episodic growth of most of the seedlings, there was no truncated seedling under 1x $2CO_2$ at d 60 as well as under 3x $2CO_2$ at d 86.

The two types of seedlings were analyzed separately. Seedlings were immediately divided into roots, stems + petioles, and leaves, which were weighed separately to estimate their fresh mass. The leaves and stems of the episodic seedlings were divided according to the different flushes. The foliar surface of every leaf was measured using an area meter (Delta-T devices, Cambridge, England), and the number of leaves was counted. Shoot length was measured in cm from root collar to terminal bud for truncated seedlings. For episodic seedlings, total shoot length was separated into two parts: from root collar to the last bud scar (previous year's growth + spring growth) and from bud scar to terminal bud (second flush of growth). The whole root, the whole stem of each flush and a sub-sample of preformed and neoformed leaves were oven-dried at 65°C for 4 d and weighed. One entire leaf from the second pair of leaves of each flush was set on ice for the immediate measurement of *in vivo* nitrate reductase (NR) activity at the field site. Three leaves from the second and third pairs of leaves of each flush were set apart for determination of wax and contact angle. The rest of the leaves were immediately set on dry ice and kept at -80°C for future enzymatic analysis.

In vivo nitrate reductase (NR) assay

At the field site, in parallel with the harvests and at all sampling dates, NR (E.C. 1.6.6.1.) activity was measured according to the method of Jaworski (1971) as modified by Truax *et al.* (1994). One hundred mg fresh weight of material from a single leaf was sampled

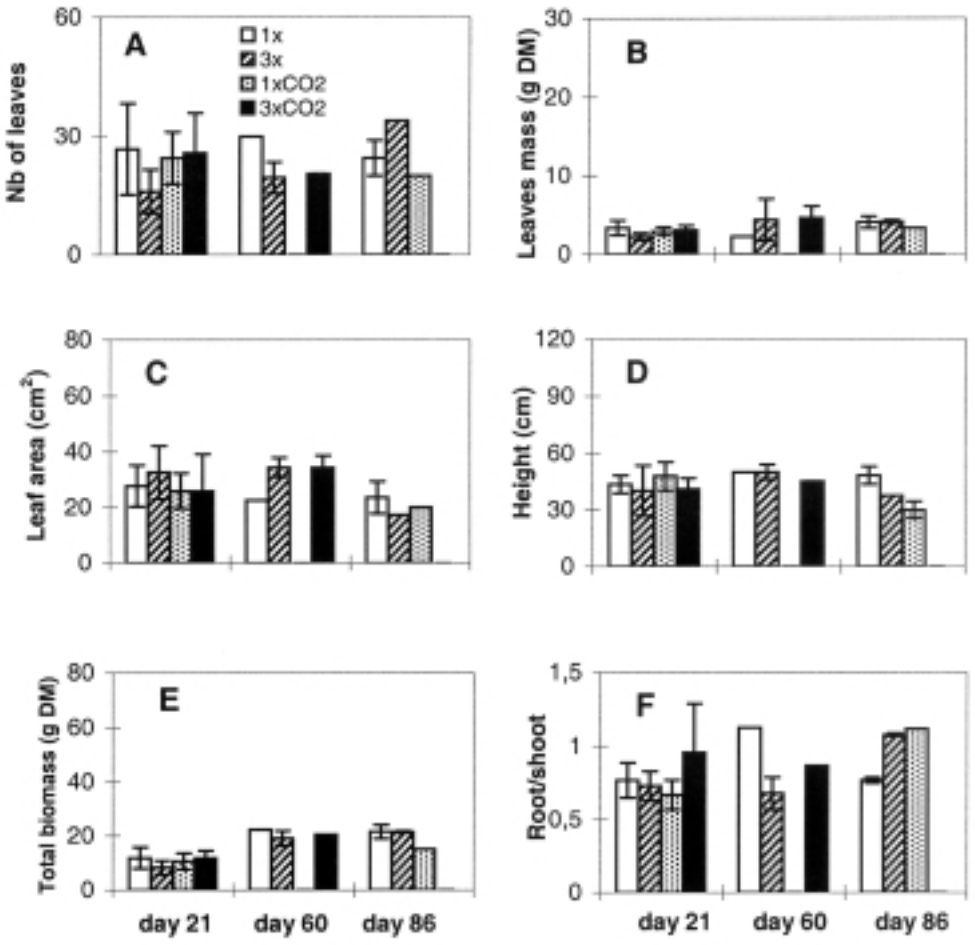

Figure 2. Number of leaves (A), biomass of leaves (B), leaf area (C), height (D), total biomass (E) and root/shoot (F) (mean ± SD) of the truncated seedlings exposed under CO_2, O_3, and their combination at days 21, 60 and 86.

from each flush of each harvested seedling, cut into 2 mm x 2 mm pieces, and incubated in 2.5 mL of 100 mM phosphate buffer (pH 7.5) containing 40 mM KNO_3 and 1.5% 1-propanol. Each sample was vortexed for 2 min to help tissue infiltration by the incubation solution. The test tubes were sealed and incubated for 1 h at 30°C. A blank containing 100 mg fresh weight of leaf material without KNO_3 was prepared for each sample. The enzymatic reaction was stopped by immersing the tubes for 5 min in boiling water. The colorimetric determination of NO_2^- was done by mixing 0.25 mL of incubation medium with 0.25 mL 0.02% N-(1-Naphthyl) ethylenediamine and 0.25 mL of sulfanilamide. After 30 min, absorbance was read at 540 nm. NR activity was not determined on neoformed leaves of the episodic seedlings of the 1x $1CO_2$ treatment at d 86.

Gas exchange

The net CO_2 assimilation rate (A) was measured between 9:30 and 12:00 in August 1995 under growing [CO_2] and high light conditions (1450 μmol m^{-2} s^{-1}) using a portable gas exchange measurement system (Li-6200, Li-Cor, Lincoln, NE, USA) equipped with a 1 L leaf chamber. The Li-6200 infrared gas analyzer was calibrated with a span gas of known [CO_2] for each measurement d. Leaf temperature was 19.2 ± 0.4°C. Measurements were done on the first pair of neoformed leaves of the episodic seedlings and were repeated seven times per leaf. After the measurements, the leaves were harvested and their surfaces were determined using an area meter (Delta-T devices, Cambridge, England).

Quantity of epicuticular waxes

For all sampling dates, wax mass was measured on three leaves of each flush of each harvested seedling according to the method of Dixon *et al.* (1997). Entire leaves were used to minimize lipid contamination from intercellular products. Leaf area was measured, then leaves were washed in chloroform for 30 sec and the chloroform solution was vacuum-filtered using cellulose nitrate filters with a pore size of 0.45 μm. The filtration removed dust particles that were on the surface of the leaves. This process was repeated three times, which ensured that all the epicuticular wax had been removed. The chloroform solution was then collected and evaporated to dryness in small preweighted bottles to allow determination of the mass of wax. Wax mass was not determined on neoformed leaves of the episodic seedlings of the 1x $1CO_2$ treatment at d 86.

Contact angles

For all sampling dates, measurements of the contact angle of a water droplet were made on the abaxial and adaxial surface of three leaves of each flush of each harvested seedling as described by Dixon *et al.* (1997). Each leaf was mounted on a microscope slide and a 1 μL drop of distilled water was placed on the leaf surface. The slide was then fixed at 90°C to the objective lens of a stereomicroscope. A specially adapted eyepiece allowed measurements of the angle between the drop and the leaf surface. This process was repeated for three drops on each leaf.

Statistical analysis

Two-way ANOVAs (proc GLM) were performed to test the effects of CO_2, O_3, and their interaction on the biomass of episodic seedlings at d 60 and 86, and on the biomass of truncated seedlings at d 21. Due to incomplete design for the truncated seedlings at d 60 and 86 (absence of truncated seedlings for the 1x $1CO_2$ treatment at d 60 and for the 3x $2CO_2$ treatment at d 86), one-way ANOVAs were performed to test the effects of the three remaining treatments on the biomass.

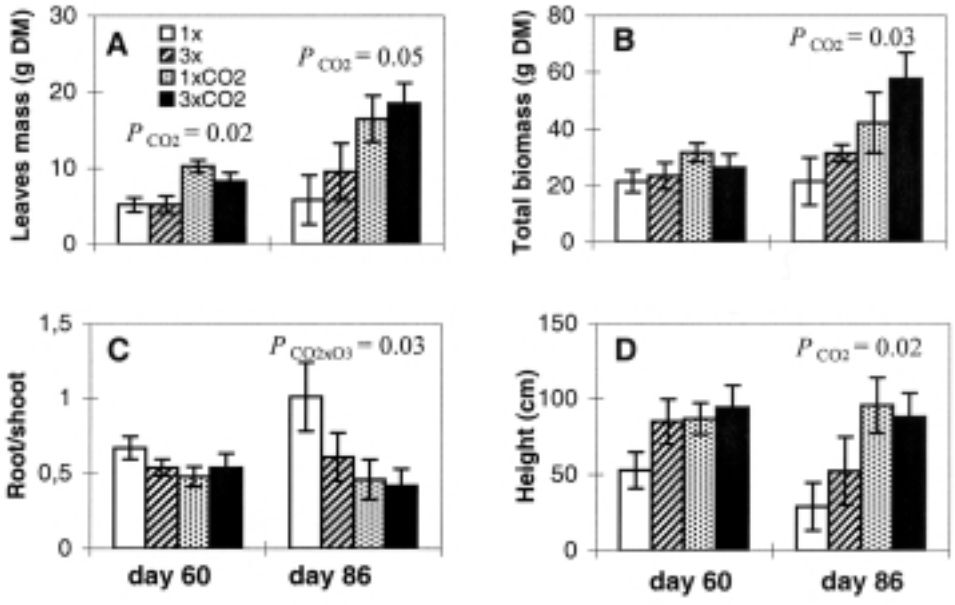

Figure 3. Leaves (A), total biomass (B), root/shoot (C) and height (D) (mean ± SD) of the episodic seedlings exposed under CO_2, O_3, and their combination at days 60 and 86. Significant effects at $P \leq 0.05$ are indicated in the figures.

Two-way ANOVAs (proc GLM) were performed to test the effects of CO_2, O_3, and their interaction on the NR activity of truncated seedlings at d 21, on the NR activity of preformed leaves of episodic seedlings at d 60 and 86, and on the NR activity of neoformed leaves of episodic seedlings at d 60. Due to incomplete design for the truncated seedlings at d 60 and 86 and for the neoformed leaves of episodic seedlings at d 86, one-way ANOVAs were performed to test the effects of the three remaining treatments on NR activity.

Two-way ANOVAs (proc GLM) were performed to test the effects of CO_2, O_3, and their interaction on the contact angles at d 60 and 86 and on the photosynthetic parameters.

Two-way ANOVAs (proc GLM) were performed to test the effects of CO_2, O_3, and their interaction on wax quantity at d 21 and 60. Due to incomplete design for the episodic seedlings at d 86, one-way ANOVA was performed to test the effects of the three remaining treatments.

The effect of time on the accumulation of biomass, NR activity, wax quantity and the contact angle of the episodic seedlings was evaluated with a multivariate analysis for repeated measures. One factor of classification was used. In the case of an incomplete design, the effect of time was evaluated for the three remaining treatments. Differences between treatments were considered significant at $P < 0.05$. Statistical tests were performed using SAS v. 6.12 (Statistical Analysis System, Cary, NC, USA).

RESULTS

Biomass

Under ambient CO_2 conditions in the open-top chamber, half of the seedlings of each O_3 treatment had an episodic growth. High [CO_2] favoured the neoformation of leaves: under 2CO_2 at 1x and 3x, respectively 87.5% and 75% of the seedlings had an episodic growth (data not shown).

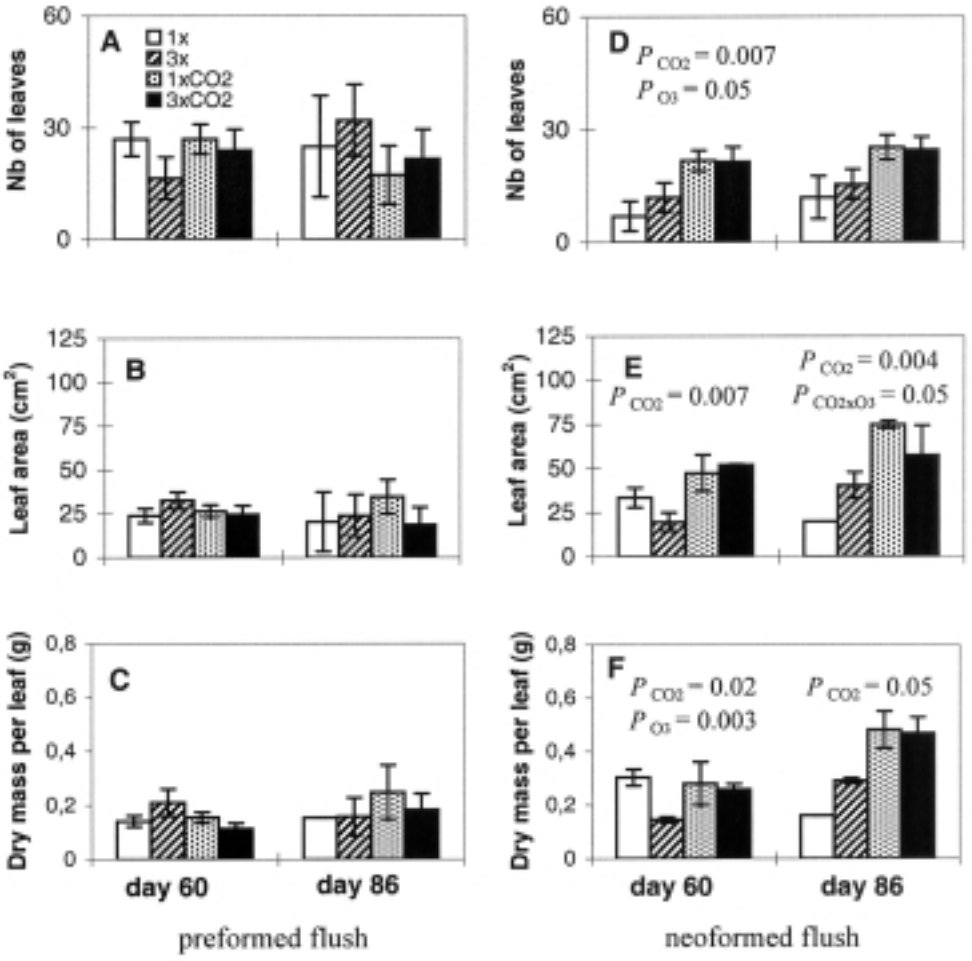

Figure 4. Number of leaves, leaf area and dry mass per leaf (mean ± SD) of the preformed (A, B, C) and neoformed (D, E, F) flushes of the episodic seedlings exposed under CO_2, O_3, and their combination at days 60 and 86. Significant effects at $P \leq 0.05$ are indicated in the figures.

Table 2. Net CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance to CO_2 (g_s, mmol m^{-2} s^{-1}), A/gs ratio and Ci/Ca ratio (intercellular CO_2 concentration/ambient CO_2 concentration) (mean ± SD) of the neoformed leaves of the episodic seedlings measured during the month of August

CO_2	Ozone	A	g_s	A/gs	Ci/Ca
350	1x	0.79 ± 0.2	21.50 ± 8.7	0.038 ± 0.004	0.84 ± 0.007
	3x	1.35 ± 0.2	28.75 ± 4.6	0.047 ± 0.001	0.80 ± 0.007
650	1x	1.39 ± 0.6	13.65 ± 6.4	1.102 ± 1.001	0.80 ± 0.01
	3x	1.92 ± 1.1	16.50 ± 7.08	0.112 ± 0.013	0.78 ± 0.04
P CO_2[a]		0.28	0.11	**0.001**	0.14
P O_3		0.44	0.35	0.232	0.09
P CO_2 x O_3		0.97	0.67	0.947	0.41

[a] *P* values are given by the two-way ANOVA. Significant effects at $P \leq 0.05$ are indicated in bold letters.

The extension of the preformed leaves of the truncated seedlings was mostly achieved at d 21. High O_3 or high CO_2 treatments did not influence the development of these truncated seedlings and no effect was observed even at the end of the season (*P* values given by the ANOVA for all variables were superior to 0.05, comparison of four or three treatments, data not shown) (Fig. 2).

Total biomass of the episodic seedlings increased with time ($P = 0.0318$; Fig. 3 A). At the end of the treatment, biomass of the seedlings grown under 3x $2CO_2$ was 37% larger than that of the 1x $2CO_2$ seedlings. The biomass of these 1x $2CO_2$ seedlings was itself 95% larger than that of the control seedlings (Fig. 3 B). Under high [CO_2], the enhanced growth of the leaves led to a root/shoot ratio around 0.5 (Fig. 3 A, C). Preformed leaves were not affected by high [CO_2] as extension was achieved early in the growing season (Fig. 4 A, B, C). However, the CO_2 fertilizing effect was important in neoformed leaves (Fig. 4 D, E, F). At d 60 and 86, larger leaf biomass of the seedlings was attributed to larger leaf area of the neoformed leaves (Figs. 3 A and 4 E).

Assimilation rate, stomatal conductance

No significant effect of the treatments was observed on net CO_2 assimilation (A) and stomatal conductance (g_s) during the month of August. However, A/g_s increased under elevated CO_2. The Ci/Ca ratio was constant and around 0.80 for all treatments (Table 2).

Nitrate reductase activity

NR activity of the preformed leaves of truncated seedlings decreased during the growing season, especially between d 21 and 60 (Fig. 5 A). NR activity in the preformed leaves of the episodic seedlings decreased significantly during the season ($P = 0.008$, Fig. 5 B). In the neoformed leaves of episodic seedlings, the level of NR activity at d 60 was higher than in the preformed leaves and was maintained at this level until the end of the season (Fig. 5 C). Moreover, under elevated CO_2, NR activity in the preformed leaves was significantly higher than under ambient CO_2 (Fig. 5).

Epicuticular wax quantity and contact angles

The epicuticular wax quantity was not affected by time ($P > 0.05$ for both flushes) or treatments during the whole growing season (Table 3). No significant changes in the contact angles were measured during the growing season ($P > 0.05$ for both flushes). There was no change in the contact angles of the leaves of both flushes of the seedlings exposed to the three treatments compared with the control seedlings (Table 4).

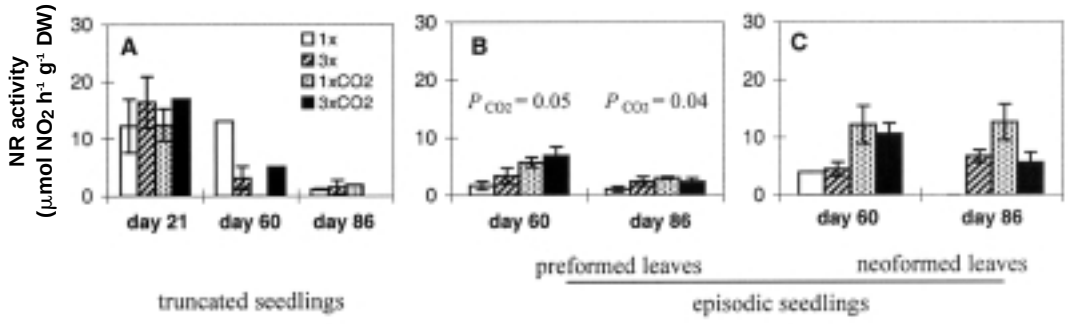

Figure 5. NR activity (μmol NO_2 h^{-1} g^{-1} DW) of the preformed leaves of the truncated seedlings (A) and of the preformed (B) and neoformed leaves (C) of the episodic seedlings exposed under CO_2, O_3, and their combination at days 21, 60 and 86. Significant effects at $P \leq 0.05$ are indicated in the figures.

Table 3. Waxes quantity (μg mm^{-2}) (mean ± SD) of the preformed and neoformed leaves of the episodic seedlings

		day 21	day 60		day 86	
CO_2	Ozone	preformed	preformed	neoformed	preformed	neoformed
350	1x	0.39 ± 0.1	0.47	0.34	=[a]	=
	3x	0.39 ± 0.1	0.49 ± 0.1	0.46 ± 0.05	0.43 ± 0.1	0.32 ± 0.04
650	1x	0.40 ± 0.1	0.48 ± 0.03	0.35 ± 0.1	0.45 ± 0.1	0.46 ± 0.2
	3x	0.35 ± 0.04	0.39 ± 0.03	0.31 ± 0.05	0.55 ± 0.04	0.46 ± 0.06
	P CO_2[a]	0.85[b]	0.95 [b]	0.83[b]	*P* = 0.48[c]	*P* = 0.72
	P O_3	0.95	0.76	0.10		
	P CO_2 x O_3	0.53	0.26	0.09		

[a] = : not determined.
[b] At days 21 and 60, *P* values reported are the result of the two-way ANOVA.
[c] At day 86, *P* values reported are the result of a one-way ANOVA.

DISCUSSION

Mature sugar maple is referred to as a fixed growth species (Kramer and Kozlowski 1979). However, in favourable environmental conditions, vigorous seedlings may develop a second flush of leaves (Gregory 1980). The environmental conditions of the study were such that no nutrient or light limitation should have occurred. Under these conditions and in ambient [CO_2], 50% of the seedlings had an episodic growth. However, under elevated CO_2 more than 75% of the seedlings had an episodic growth. For these episodic seedlings, high [CO_2] had a large fertilizer effect. In the neoformed flush of leaves, high CO_2 availability led to an increased A/g_s ratio, which may result in an increased carbon skeleton production allocated to the production of biomass. The increase in biomass in the episodic seedlings under 1x $2CO_2$ and 3x $2CO_2$ was similar to that measured by Bazzaz *et al.* (1990) after an exposure to 700 μL L^{-1} CO_2 for 100 d. However, the response of sugar maple seedlings to elevated CO_2 may vary with growth conditions. Other studies reported slight biomass increases in sugar maple seedlings in response to elevated CO_2 (Kruger *et al.* 1998; Noble *et al.* 1992). It thus seems difficult to anticipate the intensity of the growth response of sugar maple seedlings under high [CO_2].

NR activity decreased more than 3-fold during the growing season from the end of June to the end of

Table 4. Contact angle (mean ± SD) formed by a droplet of water on the adaxial and abaxial surfaces of the preformed and neoformed leaves of the episodic seedlings exposed under CO_2, O_3, and their combination at days 60 and 86

	adaxial	day 60		day 86	
CO_2	ozone	preformed	neoformed	preformed	neoformed
350	1x	97.44 ± 23.1	107.69 ± 17.7	102.56 ± 28.2	102.56 ± 3.8
	3x	76.92 ± 10.3	88.46 ± 11.5	89.74 ± 23.1	89.74 ± 9
650	1x	84.62 ± 20.5	98.72 ± 24.4	78.85 ± 23.1	91.03 ± 24.4
	3x	80.77 ± 30.8	94.23 ± 15.4	78.85 ± 20.5	89.74 ± 30.8
	P CO_2[a]	0.65	0.77	0.49	0.62
	P O_3	0.76	0.56	0.60	0.68
	P CO_2 x O_3	0.59	0.68	0.77	0.67
	abaxial	**day 60**		**day 86**	
CO_2	ozone	preformed	neoformed	preformed	neoformed
350	1x	80.77 ± 35.9	73.08 ± 10.3	84.62 ± 2.6	75.64 ± 33.3
	3x	70.51 ± 12.8	62.82 ± 17.9	70.51 ± 17.9	32.82 ± 15.4
650	1x	65.38 ± 21.8	67.95 ± 3.8	65.38 ± 21.8	60.25 ± 10.3
	3x	80.77 ± 6.4	67.95 ± 9.0	62.18 ± 20.0	66.02 ± 15.4
	P CO_2[a]	0.71	0.88	0.52	0.71
	P O_3	0.55	0.74	0.72	0.68
	P CO_2 x O_3	0.73	0.86	0.8	0.79

[a] *P* values are given by the two-way ANOVA.

August in the preformed flush of all the seedlings as its level is related to the age of the leaf tissue (Lam *et al.* 1996). A high level of NR activity was required in developing leaves because N was necessary for leaf growth. High levels of NR activity were maintained in neoformed leaves, which consist of younger tissues than preformed leaves. Under high [CO_2], a higher NR level in these leaves ensured the availability of N to sustain the enhanced development of the flush.

The 3x treatment had no effect on the net assimilation rate and growth of sugar maple seedlings under both CO_2 levels. In 1994, the United Nations Economic Commission for Europe (UN-ECE) determined a critical level of O_3 above which a 10% decrease of biomass may be expected. For trees, this level is an accumulated exposure over a threshold of 40 nL L^{-1} (AOT40) of 10 ppm h (calculated during a growing season of 6 mo, April to September, during daylight hours, under realistic [O_3]) (Fuhrer and Acherman 1994; Fuhrer *et al.* 1997). After 86 d of treatment, the seedlings of the 3x 1CO_2 treatment received a cumulative O_3 dose of 144 ppm h, which represent an AOT40 of 91.5 ppm h. Thus, the seedlings received more than the critical level. However, no decrease in biomass was measured. Moreover, some other 2-yr-old sugar maple seedlings were exposed to the same O_3 level (1x 1CO_2 and 3x 1CO_2) 1 yr before the beginning of this experiment in the same chambers. The 3x seedlings received a cumulative O_3 dose around 290 ppm h for the two growing seasons. These seedlings were harvested at the same dates and biomass accumulation at the end of the second season of exposure did not decrease compared with the control seedlings (data not shown). Similar results were reported for sugar maple seedlings by Rebbeck and Loats (1997) and Scherzer (1991) after exposure to 304 ppm h O_3 and 199 ppm h O_3 respectively over two growing seasons.

We measured a low stomatal conductance for the four treatments, as already measured on sugar maple seedlings (Gaucher *et al.* 2003; Laurence *et al.* 1996), leading to a low uptake rate of O_3 (Broadmeadow and Jackson 2000; Kolb *et al.* 1997; Pell *et al.* 1999). Thus, no irreversible damage might have occurred for sugar maple seedlings exposed to the 3x 1CO_2 treatment. Moreover, the level of fumigated O_3 fluctuated from hour to hour and from day to day. The succession of periods with low [O_3] (during cloudy days for example) and periods with higher [O_3] may allow maple seedlings to detoxify the gas and repair the damage. The detoxification processes of this species are efficient at this level of O_3 (Gaucher *et al.* 2003; Niewiadomska *et al.* 1999) and they probably allowed the seedlings to overcome the oxidative stress when present.

In another study, 2-yr-old sugar maples were exposed to a gradient of O_3 (0 to 300 nL L^{-1}) during daylight hours for a growing season of 85 d in open top chambers. Seedlings exposed to a constant concentration of 100 nL L^{-1} O_3 received a cumulative O_3 dose of 118 ppm h and an AOT40 of 70 ppm h, which is lower than in the present study. However, the biomass of the seedlings decreased by 48% compared with the control. The light, water and nutrient supplies were similar in both studies. Thus, for sugar maple seedlings, a constant level of fumigation during daylight hours was more damaging than the fluctuating O_3 level, even when receiving similar cumulative O_3 doses.

Few studies examined epicuticular waxes on deciduous species (Garrec *et al.* 1995; Karnosky *et al.* 1999; Kerfourn and Garrec 1991). A decrease in the contact angle indicates a more hydrophilic cuticular surface due to the deposition of more hydrophilic substances on the leaf surface or to the degradation of the epicuticular wax structure (Cape 1983). Following O_3 exposure, a change in the chemical composition and micromorphology of the waxes, which become less hydrophobic, leads to an increase in the wettability of the leaf surface. This may have many consequences such as enhancing foliar leaching and increasing the rates of cuticular transpiration and pollutant uptake (Barnes *et al.* 1988). In parallel, the occlusion of the stomata by an amorphous layer of degraded waxes may have physiological consequences such as decreasing photosynthesis, stomatal transpiration and O_3 uptake. In the present study, the contact angles of both leaf surfaces did not decrease in response to the treatments and no significant decrease in wax quantity was measured. Thus, the cuticule was not damaged by the oxidative conditions of the treatments and conserved its protective properties.

Under the O_3 fumigation level used in this experiment, O_3 had no negative impact on the development of young sugar maple seedlings during one growing season. When CO_2 and O_3 were fumigated together, O_3 did not decrease the fertilization effect of high [CO_2]. We therefore conclude that sugar maple seedlings will benefit from the [CO_2] increase at least until the end of this century providing that soil nutrient supplies are sufficient. They may thus face competition in their first yr of growth.

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