# Interactive Effects of Elevated Ozone plus Carbon Dioxide on Duckweeds Exposed in Open-Top Chambers[1]

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ABSTRACT. **The response of *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleiden to projected future ambient levels of $O_3$ and $CO_2$ was studied under field conditions. The two duckweed species were treated with either charcoal-filtered air (CF), ambient $O_3$ ($1XO_3$), twice ambient $O_3$ ($2XO_3$), twice ambient $CO_2$ plus twice ambient $O_3$ ($2XCO_2+2XO_3$), or chamberless open-air (OA). Two experiments were conducted. In Experiment I, *L. minor* was treated for 15 d with a cumulative $O_3$ exposure of 14.4 ppm· h. No $O_3$ effects were observed during Experiment I. Dry weight of individual fronds and photosynthesis per frond increased in *L. minor* exposed to $2XCO_2+2XO_3$- air. In Experiment II after 25 d of treatment (cumulative $O_3$ exposure of 16.2 ppm h), negative effects of $2XO_3$ on the photosynthetic and growth rates of *L. minor* were observed. Dark respiration of *L. minor* significantly increased in $2XO_3$-air compared with controls, but declined significantly in $2XCO_2+2XO_3$-air compared to those grown in $2XO_3$-air. Photosynthesis and dry weight per frond increased in $2XCO_2+2XO_3$-air when compared with all other treatments. Measurement of $A/C_i$ (assimilation versus intercellular $CO_2$ concentration) curves in *L. minor* showed a significant reduction in carboxylation efficiency and maximum rates of photosynthesis in $2XCO_2+2XO_3$-air compared with other treatments when expressed per weight. No differences in carboxylation efficiency were detected between treatments when expressed per frond.**

**After 25 d of treatment, photosynthesis (per frond) and dry weight of *S. polyrhiza* were reduced in $2XO_3$-air, but final frond number was unaffected. Dark respiration of *S. polyrhiza* was unaffected in $2XO_3$-air, but when exposed to $2XCO_2+2XO_3$-air, it declined significantly. Although *S. polyrhiza* photosynthesis per frond increased in $2XCO_2+2XO_3$-air, dry weight was unaffected when compared with all other treatments. Only when comparisons were made between *S. polyrhiza* grown in $2XCO_2+2XO_3$-air and $2XO_3$-air, were significant increases in dry weight observed. The addition of $2XCO_2$ to $2XO_3$-air resulted in amelioration of negative $O_3$ effects for most responses for both duckweed species.**

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## INTRODUCTION

Increasing atmospheric concentrations of carbon dioxide ($CO_2$) and ozone ($O_3$) have prompted numerous studies evaluating the single effects of these gases on plant growth and physiology. However, few studies have investigated the interactive effects of elevated $O_3$ and elevated $CO_2$. Generally, elevated $CO_2$ concentrations have been shown to increase photosynthetic rates (Campbell and others 1988; Bowes 1991; Baker and Allen 1994) and reduce respiration rates (Baker and Allen 1994). However, Socias and others (1993) did report a decrease in photosynthesis of *Phaseolus vulgaris* exposed to high $CO_2$. Above ambient concentrations of $O_3$ have been shown to inhibit photosynthesis (MacDowall 1965; Black and others 1982; Forberg and others 1987; Hanson and others 1988; Kramer and others 1991; Aarnes and others 1993; Edwards and others 1994), while dark respiration has been either unaffected (Black and others 1982; Hanson and others 1988; Aarnes and others 1993) or stimulated by high $O_3$ (Rowland-Bamford and others 1989; Coleman and others 1995).

Conflicting effects of $O_3$ on growth rates have also been reported. Ozone in low concentrations may be stimulatory or have no effect (Bennett and others 1979; Edwards and others 1994), while high concentrations may be inhibitory (Darrall 1989; Jensen 1981). Plant mass is often reduced by exposure to high $O_3$ concentrations (Bennett and others 1979; Ito and others 1985; Edwards and others 1994). Biochemical changes within the chloroplasts often occur after exposure to environmental gases. High $O_3$ (Sasek and Richardson 1989) and high $CO_2$ (Van Oosten and others 1992) have been shown to reduce ribulose bisphosphate carboxylase/oxygenase (Rubisco) activity and ribulose bisphosphate (RuBP) regeneration. In $CO_2$ x $O_3$ interaction studies, enriched $CO_2$ can alleviate the negative effects of $O_3$ on plant photosynthesis and growth (Krupa and Kickert 1989; Kramer and others 1991; Mulchi and others 1992). The ameliorative effect of elevated $CO_2$ may be the result of increased photosynthesis or stomatal closure, which would decrease $O_3$ entry into the leaves. Others (Rudorff and others 1996) found no interactions between elevated $O_3$ and elevated $CO_2$ on the yield of wheat or corn grown in open-top chambers.

Most of the gaseous pollutant plant response work has been conducted on crops or tree seedlings with less attention on aquatic plants such as duckweeds. Duckweeds

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are members of the Lemnaceae family and provide special advantages for physiological study which include: rapid asexual propagation, small size, and ease of culture on chemically-defined media. Previous work suggests duckweeds have continuously open stomata which provides the opportunity to investigate effects of air pollutants on leaf physiology without complications of guard cell behavior (Bauer and others 1976; Park and others 1990). Short-term exposure to high $CO_2$ has been shown to stimulate photosynthesis in duckweeds (Loats and others 1981). In *L. gibba* 1-2 h acute $O_3$ exposures, ranging from 150 to 600 ppb, reduced photosynthesis (Forberg and others 1987; Aarnes and others 1993). Dark respiration was decreased in *L. gibba* by $O_3$, but only when the mean $O_3$ concentrations exceeded 300 ppb (Aarnes and others 1993). Prior duckweed studies have investigated effects of short-term $CO_2$ and/or acute exposures to high concentrations of $O_3$ under controlled laboratory conditions. Diminished sensitivity is often reported when plants are exposed to $O_3$ in open-top chambers compared with those in growth chambers or greenhouses (Lewis and Brennan 1977). Information is needed to understand how aquatic angiosperms respond to gaseous pollutants when grown out-of-doors. The primary goal of this study was to determine the response of two species of duckweed to projected future ambient levels of $O_3$ and $CO_2$ under realistic field conditions as measured by growth and gas exchange.

## MATERIALS AND METHODS

Two experiments were conducted in late summer and early autumn of 1993. Exposures were conducted in standard outdoor, open-top chambers (3m-diameter) at the USDA Forest Service Laboratory near Delaware, OH. Carbon dioxide and $O_3$ fumigation occurred continuously in three replicates of five treatments: 1) charcoal-filtered air (CF); 2) ambient $O_3$ air ($1XO_3$); 3) twice ambient $O_3$ air ($2XO_3$); 4) twice ambient $CO_2$ plus twice ambient $O_3$ air ($2XCO_2+2XO_3$); or 5) open-air chamberless plot (OA). Twice ambient $CO_2$ (700 ppm) was supplied by vaporization of liquid $CO_2$ (MG Industries, Pennsauken, NJ). Ozone was produced from pressurized, dry oxygen using a silent-arc generator (OREC Model 03V10). The concentrations of both $CO_2$ and $O_3$ were monitored and recorded every 3-4 min with either a LICOR $CO_2$ analyzer (Model 6252) or a TECO $O_3$ meter (Model 49PS). Treatment, dispensing, and monitoring systems are described elsewhere (Rebbeck 1996).

Two duckweed species, *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleiden (collected in Granville Township, Licking County, OH), were cultured in 250 mL beakers on 125 mL of modified, sucrose free, Hoagland's medium (Loats and others 1981). In each of two experiments, the duckweeds were transferred from actively multiplying stock cultures. After placement in the center of each open-top chamber, individual beakers were covered with 40% nylon shade screen to reduce heat, discourage insect contamination, and allow for adequate air exchange. Since $O_3$ levels were low at the soil surface within the chambers, the beakers were elevated to 0.5 m. In Experiment I, approximately three plants (a total of 8-13 fronds) were initially transferred to each beaker (n = 9, three replicates/chamber) and counted every 3-4 d. At the start of Experiment II there were approximately 4 plants per beaker with a total of 13-17 fronds. On the final day of fumigation, net photosynthesis, dark respiration, and/or $CO_2$ response curves were determined with a LICOR 6200 portable photosynthesis system. All of the plants, from within a 250 mL beaker, were spread on moistened filter paper and placed in an open petri dish (8 x 90 mm) to allow for adequate gas exchange. After 10 min of equilibration, the plant sample was placed in a 1-L LICOR cuvette and illuminated at 800 $\mu mol\ m^{-2}\ s^{-1}$ PAR (photosynthetically active radiation) using a GE 300 W.120V. cool beam, wide flood lamp. This light level was comparable to that observed within the open-top chambers. After net photosynthesis was measured, plants were equilibrated in darkness for 2 min before dark respiration was measured. In Exp. II, the relationship between net assimilation (A) and internal $CO_2$ concentration ($C_i$) ($A/C_i$) in *L. minor* was determined at 800 $\mu mol\ m^{-2}\ s^{-1}$ PAR, by measuring net photosynthesis sequentially at 900, 700, 350, 250, 150, 100, and 50 ppm ambient $CO_2$ with a 2.5 min equilibrium prior to each measurement. Analysis of $A/C_i$ relationships was done using similar methods reported by Sasek and Richardson (1989). The initial slope was calculated and used as an estimate of carboxylation efficiency; and regeneration of RuBP was estimated by the saturated rate of net photosynthesis. Immediately following gas exchange measures, the duckweeds were oven-dried at 75° C for 48 h to determine dry weights.

To minimize algal growth, the medium was diluted in each experiment and changed weekly. In Experiment I, *L. minor* was cultured on 1/12X growth medium. In Experiment II, both *S. polyrhiza* and *L. minor* were cultured on 1/20X growth medium. The data were evaluated by ANOVA or GLM to test for treatment effects using the SAS statistics program (SAS 1988). Chamber means were used in all analyses.

## RESULTS

### Treatment exposure and growth environment

A summary of the environmental conditions ($O_3$ levels, relative humidity, air temperature, PAR [photosynthetically active radiation] and rainfall) during each experiment is shown in Table 1. During Experiment I, the 8-h (1000-1700 h EST) mean $O_3$ concentration was 63, 44, 40, and 6 ppb for $2XO_3$-, $1XO_3$-, ambient- and CF-air, respectively. The mean $CO_2$ concentrations were 723 and 366 ppm for $2XCO_2$- and ambient $CO_2$-air, respectively. During Experiment II, the 8-h mean $O_3$ concentration was 53, 47, 34, 26, and 8 ppb for $2XCO_2$+ $2XO_3$-, $2XO_3$-, $1XO_3$-, ambient- and CF-air, respectively. The mean $CO_2$ concentration was 670 and 364 ppm, for $2XCO_2$ and ambient $CO_2$, respectively. Daily peak ambient and $2XO_3$ concentrations for each experiment are shown in Figure 1. Experiment I had 5 days with peak $O_3$ values exceeding 60 ppb, while Experiment II had one day exceeding 60 ppb $O_3$. Mean daily air temperature was about 6° C lower during the second experiment than the first experiment.

TABLE 1

*Environmental conditions (mean ± std deviation) during Experiment I (26 August–9 September 1993) and Experiment II (9 September–2 October 1993) in Delaware, OH.*

| | | | Experiment I | Experiment II |
|---|---|---|---|---|
| A. Ozone* | | | | |
| Target | Actual | | | |
| | EXP I | EXP II | | |
| Ambient | – | – | 40.3 ± 11.4 | 25.9 ± 10.5 |
| CF | 0.14X | 0.31X | 5.7 ± 2.4 | 8.0 ± 2.8 |
| 1X $O_3$ | 1.10X | 1.30X | 44.4 ± 11.7 | 33.7 ± 14.0 |
| 2X $O_3$ | 1.54X | 1.80X | 62.2 ± 19.1 | 46.7 ± 24.3 |
| 2X $O_3$ + 2X$CO_2$ | 1.56X | 2.03X | 62.8 ± 20.1 | 52.7 ± 48.7 |
| B. Environmental Variables | | | | |
| PAR (mol $m^{-2}$ $day^{-1}$)† | | | 0.139 | 0.169 |
| Relative humidity 1000–1400h (% rh) | | | 57.3 ± 8.6 | 67.7 ± 11.2 |
| Mean air temperature (°C) | | | 22.36 ± 3.37 | 16.40 ± 3.11 |
| Maximum air temperature (°C) | | | 29.27 ± 4.30 | 20.96 ± 4.41 |
| Minimum air temperature (°C) | | | 15.53 ± 3.30 | 9.38 ± 4.17 |
| Total rainfall (cm) | | | 6.78 | 6.99 |

*Mean $O_3$ concentration (ppb) ± standard deviation during 1000–1700 h each day.
†Sum of photosynthetically active radiation during experiment.

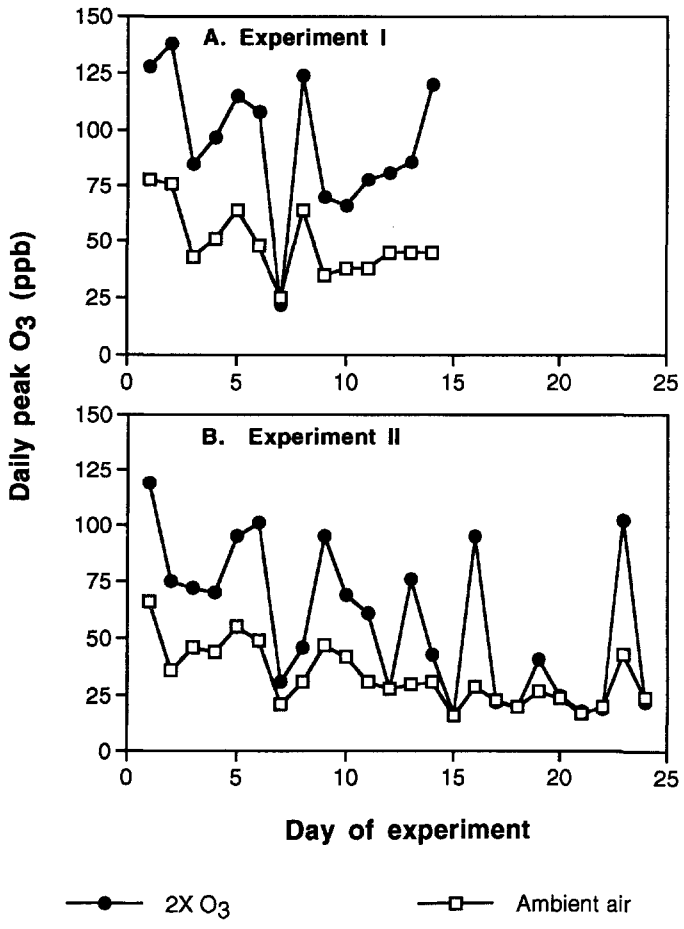


FIGURE 1. Daily peak $O_3$ concentrations in ambient air (AA) and 2X$O_3$ plots during a (A) Experiment I (15 d) from 26 August–9 September 1993; and (B) Experiment II (25 d) from 9 September–2 October 1993 in Delaware, OH.

### *Lemna minor*

After 15 d of exposure (Exp. I), no effect of fumigation on photosynthesis was observed, when expressed on a dry weight basis (Table 2). Dry weight ($p = 0.005$) and photosynthesis per frond ($p = 0.006$) increased significantly in 2X$CO_2$+2X$O_3$-air, compared with all other treatments. Final frond count per flask in Exp. I was not affected by the treatments (Table 2).

After 25 d of exposure (Exp. II), 2X$O_3$-air significantly reduced final frond number of *L. minor* compared with those grown in CF- and 1X$O_3$-air (Table 2). Photosynthetic inhibition in 2X$O_3$-air was only observed when expressed per individual frond. Dry weight was significantly reduced in plants exposed to 2X$O_3$-air when compared with all other treated plants. Photosynthesis was not affected by $O_3$ or 2X$CO_2$+2X$O_3$ when expressed on a dry weight basis. Fumigation with 2X$CO_2$+2X$O_3$-air significantly increased frond dry weight ($p = 0.0003$) and photosynthesis per frond ($p = 0.007$). Twice ambient $O_3$-air increased dark respiration, while 2X$CO_2$+2X$O_3$-air decreased rates on a dry weight basis ($p = 0.0001$). A/$C_i$ curves generated for *L. minor*, indicated that treatment with 2X$CO_2$+2X$O_3$-air decreased carboxylation efficiency 46% ($p = 0.0005$) and maximum rates of photosynthesis 55% ($p = 0.0003$) compared with all other treatments when expressed per dry weight (Fig 2). However, no treatment differences in carboxylation efficiency were detected when expressed per frond ($p = 0.467$). Twice ambient $O_3$- air did not impact these estimates of enzymatic photosynthetic activity.

### *Spirodela polyrhiza*

Following 25 d of fumigation (Exp. II), final frond count ($p = 0.214$) was not impacted by elevated $O_3$ or

TABLE 2

*Growth and physiological means (±SE) and probability values for* Lemna minor *L. treated with ozone and elevated carbon dioxide for 15 d (Experiment I) or 25 d (Experiment II).*

| Treatment | Final frond count per flask | | Dry weight per frond (mg) | | Photosynthesis per mg DWT* | | Photosynthesis per frond | | Respiration per mg DWT* | Respiration per frond |
|---|---|---|---|---|---|---|---|---|---|---|
| | Expt I | Expt II | Expt I | Expt II | Expt I | Expt II | Expt I | Expt II | Expt II | Expt II |
| CF | 237 (33) | 270 ac (36) | 0.143 a (0.002) | 0.159 a (0.014) | 2.359 (0.285) | 1.790 (0.043) | 0.213 a (0.030) | 0.180 a (0.015) | -0.592 a (0.021) | -0.058 ab (0.007) |
| $1XO_3$ | 197 (33) | 330 a (15) | 0.134 a (0.019) | 0.144 a (0.006) | 2.849 (0.066) | 1.760 (0.069) | 0.239 a (0.028) | 0.154 ab (0.018) | -0.577 a (0.032) | -0.041 c (0.012) |
| $2XO_3$ | 261 (26) | 194 b (10) | 0.142 a (0.005) | 0.122 b (0.007) | 2.207 (0.186) | 1.902 (0.265) | 0.208 a (0.014) | 0.144 b (0.014) | -0.908 b (0.030) | -0.067 b (0.004) |
| $2XO_3 + CO_2$ | 283 (10) | 246 bc (73) | 0.207 b (0.002) | 0.202 c (0.007) | 2.496 (0.028) | 1.74 (0.25) | 0.327 b (0.000) | 0.220 c (0.024) | -0.273 c (0.035) | -0.036 a (0.006) |
| p-value† | 0.167 | 0.021 | 0.005 | 0.000 | 0.125 | 0.765 | 0.006 | 0.007 | 0.000 | 0.069 |

* Photosynthesis and respiration are expressed as μmol $CO_2$ $s^{-1}$ $mg^{-1}$ DWT.
†ANOVA was done on chamber means (n = 3 per treatment) to test for significant treatment effects.

$CO_2$+$O_3$-air (Table 3). Frond dry weight was significantly reduced in duckweeds grown in $2XO_3$-air compared with those from all other treatments (p = 0.034). Photosynthesis was not affected by $O_3$ or $CO_2$+$O_3$ when expressed on a dry weight basis (p = 0.356). However, on a frond basis, fumigation with $2XCO_2$+$2XO_3$-air significantly increased photosynthesis compared with the rates of those treated with $1XO_3$- or $2XO_3$-air (p = 0.011). Dark respiration was not significantly affected in $2XO_3$-air. In $2XCO_2$+$2XO_3$-air, dark respiration was significantly inhibited, when expressed per dry weight (p = 0.0002) or frond (p = 0.019).

## DISCUSSION

Differences in the response of *L. minor* to $O_3$ were observed between Experiments I and II. No significant growth or physiological impacts from $O_3$ were observed during Experiment I. Although the daily 8-h mean $O_3$ concentration in twice ambient $O_3$-air was higher in Experiment I (63 ppb $O_3$) than Experiment II (50 ppb $O_3$), the cumulative $O_3$ exposure was greater in Experiment II (16.2 ppm h) than Experiment I (14.4 ppm h). The dynamics of $O_3$ exposures can impact the magnitude of the response. Forberg and others (1987) reported that net photosynthesis in *Lemna gibba* was reduced more by a shorter exposure to high $O_3$ levels than by a longer exposure to a lower concentration of $O_3$. However, we did not observe that response. The response of terrestrial plants to $O_3$ is greatly influenced by a number of environmental and cultural factors. Ozone damage is often greater in well-watered, fertilized, fast growing plants (Winner 1994). Photosynthesis and biomass production (mass produced per day) were lower (across treatments) in Experiment II. This was expected since light intensity and duration, ambient air temperature, and nutrient levels were lower. Filbin and Hough (1985) report seasonal variations in photosynthesis of *L. minor* correlate principally with temperature and light intensity. Based on the response of herbaceous crop plants and trees, we would have predicted that these plants would have been less sensitive to $O_3$ but the contrary was observed.

Elevated $O_3$ has been shown to decrease biomass in many herbaceous and woody species (Bennett and others 1979; Ito and others 1985; Rowland-Bamford and others 1989; Matyssek and others 1993; Edwards and others 1994). In Experiment II, biomass decreased 20-23% in both duckweed species with increasing exposure to $O_3$. For *L. minor*, this was also accompanied by decreases in photosynthesis ($frond^{-1}$) and percent frond number in $2XO_3$-air. Aarnes and others (1993) reported similar responses with *L. gibba*. Although dry weight per frond of *S. polyrhiza* was reduced by fumigation with $2XO_3$-air compared with CF-air, percent change in frond number was unaffected. Since dry weights were lower in $2XO_3$-air, photosynthetic rates, when expressed on a dry weight basis, were inflated. Consequently, expressing photosynthesis on a frond basis, which did decrease with increasing $O_3$ in both species, may be more reflective of $O_3$ effects on duckweeds. In most $O_3$ sensitive species, photosynthetic rates decrease as the $O_3$ concentration increases (Forberg and others 1987; Darrall 1989; Rowland-Bamford and others 1989; Kramer and others 1991; Aarnes and others 1993; Edwards and others 1994).

The effect of $O_3$ on dark respiration varied with species of duckweed. For *S. polyrhiza*, dark respiration rates were unaffected by exposure to $O_3$ alone, while for *L. minor*, rates were significantly increased in twice ambient $O_3$. MacDowall (1965) found an initial inhibition

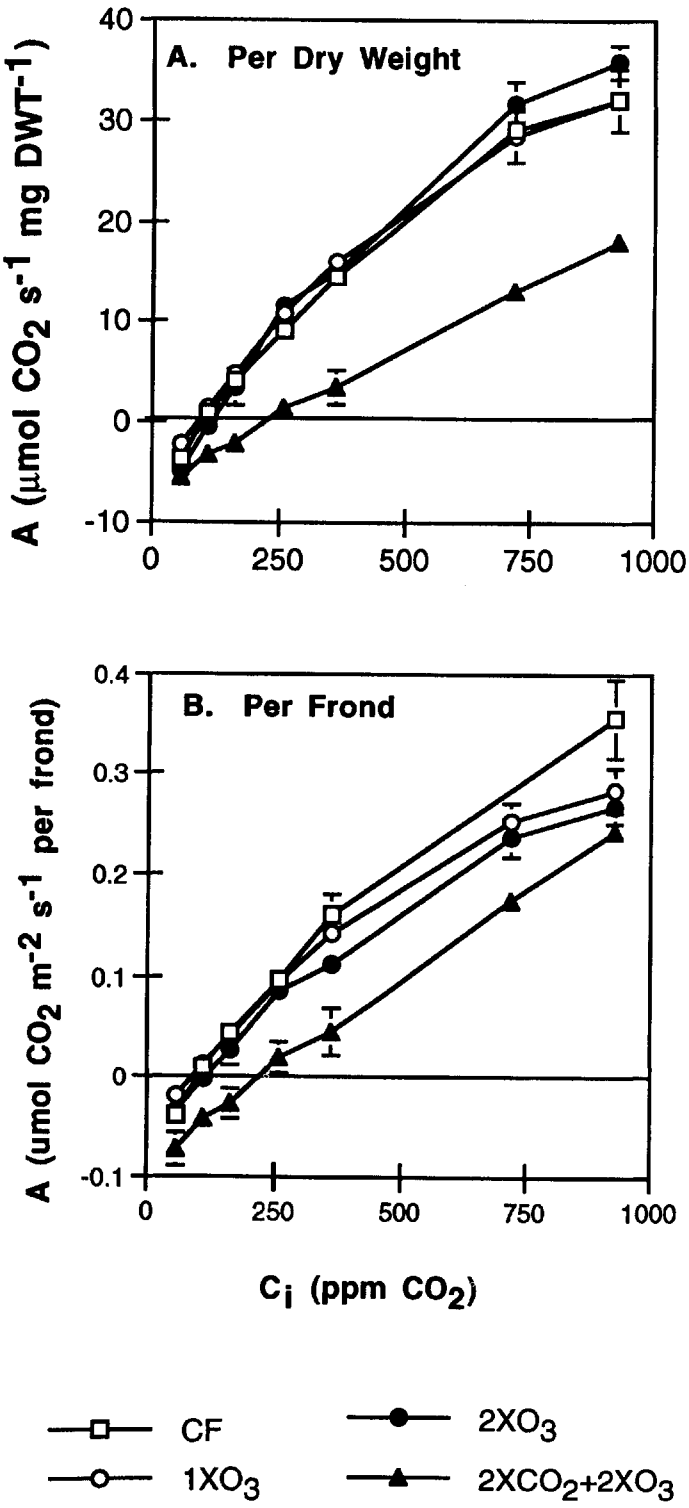


FIGURE 2. Carbon dioxide (A/Ci = assimilation vs. intercellular $CO_2$ concentration) response curves of *Lemna minor* L. exposed to elevated $O_3$ and $CO_2$ for 25 d expressed: A) per dry weight; and B) per frond.

of dark respiration in tobacco leaves fumigated with high $O_3$, but oxygen uptake was stimulated as soon as visible $O_3$ damage appeared on the leaves. Other crop plants exposed to $O_3$ show increases in dark respiration rates (Myhre and others 1988; Rowland-Bamford and others 1989). Aarnes and others (1993) however, found that dark respiration of *L. gibba* was reduced only after being exposed to relatively high $O_3$ levels. In the current study, dark respiration was inhibited in both species in $2XCO_2+2XO_3$-air. Dark respiration rates have been reported to decline in plants exposed to elevated $CO_2$ (Baker and Allen 1994; Drake and Gonzalez-Meler 1997).

Based on $A/C_i$ curves of *L. minor* (expressed per frond), carboxylation efficiency and maximum photosynthesis were not impacted by exposure to $O_3$ in our experiment. A study by Roper and Williams (1989) also found no significant difference in $A/C_i$ curves for grapes fumigated with ambient $O_3$ when compared with CF-air treated plants. However, Kull and others (1996) report that $O_3$-sensitive and $O_3$-tolerant aspen clones both showed decreased carboxylation efficiency and maximum photosynthesis when exposed to $O_3$ at ambient $CO_2$ levels. In the $O_3$- tolerant clone, exposures to $O_3+CO_2$ decreased the $A/C_i$ response curve more than $O_3$ alone. In our present study, duckweeds treated with $2XCO_2+2XO_3$-air had significantly lower $A/C_i$ curves than other treatments (only when expressed per dry weight). Under long-term exposure to high $CO_2$, less Rubsico is produced while in the presence of $O_3$ more Rubisco is degraded, and carboxylation efficiency is reduced (Sasek and Richardson 1989; Van Oosten and others 1992). Lower maximum photosynthesis rates of high $CO_2$-exposed plants may be due to a reduction in the regeneration of RuBP (Van Oosten and others 1992).

In this study, when $CO_2$ concentrations were doubled to 700 ppm, most of the negative effects of high $O_3$ concentrations appear to have been ameliorated in both duckweed species. Dry weight and photosynthesis per frond of *L. minor* increased in $2XCO_2+2XO_3$-air in both experiments. Similarly, photosynthesis per frond of *S. polyrhiza* increased following fumigation with $2XCO_2+2XO_3$-air. Kramer and others (1991) reported when soybeans were exposed to 500 ppm $CO_2$, the detrimental effects of $O_3$ on photosynthesis and dry weight were counteracted. Mulchi and others (1992), also reported an amelioration of $O_3$ by $CO_2$ in soybeans fumigated with elevated $O_3$ and $CO_2$ concentrations. The findings in our investigation indicate that increasing $CO_2$ concentrations may ameliorate negative effects of elevated $O_3$ in aquatic plants. Terrestrial plant response studies suggest that the ameliorative effects result from decreases in stomatal conductance, which is commonly induced by high $CO_2$ levels. This a plausible explanation for many crop and woody species which have responsive stomata, but for duckweeds, which have continuously open stomata (Bauer and others 1976; Park and others 1990), some other mechanism must be involved, such as shifts in secondary plant metabolism. To better understand the interactive effects of $O_3$ and $CO_2$, further investigation is required.

Our study both supports and conflicts with earlier work on the response of duckweed (Beer 1985; Forberg and others 1987; Aarnes and others 1993) to elevated $CO_2$ and/or $O_3$. Overall we observed an amelioration of negative $O_3$ effects in $CO_2$-enriched air of *L. minor* and *S. polyrhiza*. Additional experiments are needed to determine the influence of other environmental factors, such as temperature, nutrient supply, and light intensity

TABLE 3

*Growth and physiological means (±SE) and probability values for* Spirodela polyrhiza *L. Schleiden treated with ozone and elevated carbon dioxide for 25 d (Experiment II).*

| Treatment | Final frond count per flask | Dry weight per frond (mg) | Photosynthesis per mg DWT* | Photosynthesis per frond | Respiration per mg DWT* | Respiration per frond |
|---|---|---|---|---|---|---|
| CF | 168 (5) | 0.389 b (0.016) | 2.373 (0.098) | 0.577 a (0.044) | -0.606 a (0.015) | -0.166 a (0.021) |
| $1XO_3$ | 171 (4) | 0.374 b (0.015) | 2.279 (0.110) | 0.532 ab (0.015) | -0.662 a (0.008) | -0.155 a (0.005) |
| $2XO_3$ | 158 (15) | 0.312 a (0.009) | 2.271 (0.096) | 0.446 b (0.026) | -0.646 a (0.058) | -0.122 ab (0.012) |
| $2XO_3 + CO_2$ | 189 (8) | 0.417 b (0.033) | 2.760 (0.362) | 0.722 c (0.075) | -0.317 b (0.004) | -0.081 b (0.007) |
| p-value† | 0.214 | 0.034 | 0.356 | 0.011 | 0.000 | 0.019 |

*Photosynthesis and respiration are expressed as μmol $CO_2$ $s^{-1}$ $mg^{-1}$ DWT.
†ANOVA was done on chamber means (n = 3 per treatment) to test for significant treatment effects.

and duration on the response of duckweeds to increases in these atmospheric gases. This is especially important for the potentially interacting effects of temperature and light intensity, which are primary controlling factors of photosynthesis in *L. minor* in field conditions (Filbin and Hough 1985). Additional insights might be provided by conducting studies to investigate seasonal effects on duckweed response to gaseous air pollutants.

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