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Effects of decadal exposure to interacting elevated CO_2 and/or O_3 on paper birch (*Betula papyrifera*) reproduction

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In this study, we found that elevated CO_2 enhances and elevated O_3 decreases birch reproduction and early seedling growth.

Abstract

We studied the effects of long-term exposure (nine years) of birch (*Betula papyrifera*) trees to elevated CO_2 and/or O_3 on reproduction and seedling development at the Aspen FACE (Free-Air Carbon Dioxide Enrichment) site in Rhinelander, WI. We found that elevated CO_2 increased both the number of trees that flowered and the quantity of flowers (260% increase in male flower production), increased seed weight, germination rate, and seedling vigor. Elevated O_3 also increased flowering but decreased seed weight and germination rate. In the combination treatment (elevated $CO_2 + O_3$) seed weight is decreased (20% reduction) while germination rate was unaffected. The evidence from this study indicates that elevated CO_2 may have a largely positive impact on forest tree reproduction and regeneration while elevated O_3 will likely have a negative impact.
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1. Introduction

Global increases in air pollutants pose a major threat to the functioning, structure and diversity of natural and semi-natural ecosystems (Bobbink, 1998). Damaging O_3 concentrations currently occur over 29% of the world's temperate and subpolar forests but are predicted to affect fully 60% by 2100 (Fowler et al., 1999; Ryerson et al., 2001). Simultaneously, background concentrations of greenhouse gases such as CO_2 are increasing (IPCC, 2007). Atmospheric CO_2 concentration is expected to rise from a current 372 μmol mol^{-1} to about 550 μmol mol^{-1} by the middle of the century (IPCC, 2007; Prentice, 2001).

Paper birch (*Betula papyrifera* Marsh.) is one of the most widely distributed tree species in North America. Aspen (*Populus tremuloides* Michx.), maple (*Acer saccharum* Marsh.) and birch comprise 50% of the U.S. Lake States (Minnesota, Wisconsin and Michigan) forest resources and nearly 70% of all the pulpwood harvested in the Great Lakes region is aspen and birch (Piva, 1996). Birch is a pioneer species which co-occurs with aspen and is an important pulpwood, lumber, and firewood species. It is also a significant wildlife habitat species and ornamental landscape tree, and contributes spectacularly to fall color.

Ward and Strain (1997) predicted that elevated CO_2 will affect fundamental physiological and molecular processes

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and thus will impact both growth and reproductive responses of plants. Elevated CO_2 has been shown to increase reproductive potential (flowering and fruit and seed production) of plants (Garbatt and Bazzaz, 1984; Curtis et al., 1994, 1996; Idso and Kimball, 1997; Ward and Strain, 1997; Johnson and Lincoln, 2000; Edwards et al., 2001; Bunce, 2005). Early reproductive maturity has also been reported, likely due to a disproportionate carbon allocation to reproduction (Ward and Strain, 1999).

Long-term exposure of trees to elevated CO_2 has been shown to result in earlier reproductive maturation and increased cone and seed production in *Pinus taeda* L. (loblolly pine) (Ladeau and Clark, 2006a,b). Jablonski et al. (2002) reported enhanced numbers of flowers, fruits, seeds, individual seed mass, total seed mass, and decreased seed nitrogen concentration under elevated CO_2. It has been further reported that elevated CO_2 increased seed weight, lipid content and germination success in *Silen latifolia* (Wang, 2005).

The chemistry of seeds and seedling development has also been reported to be altered in pines grown under elevated CO_2 (Hussain et al., 2001) as seeds developed in elevated CO_2 had 91% and 256% greater weight and lipid content, respectively, three times the germination success, and an earlier germination time compared to those developed in current ambient CO_2. Germination is an energy demanding process that is largely fueled by mobilization and oxidation of storage lipids.

There is considerable evidence from the literature that exposure to O_3, even at current ambient levels in many regions of the world, reduces seed and fruit yields and adversely affects seed quality (Black et al., 2000). Stewart (1998) showed that exposure to realistic O_3 episodes at developmental stages can influence the reproductive structures directly, promoting significant adverse effects on floral development, seed yield and quality, and seedling vigor. Chappelka (2002) reported that O_3 accelerated and increased initial floral development but delayed fruit development as well as decreased seed size in blackberry (*Rubus cuneifolius*). It has been observed that O_3 caused flower bud abortion in *Brassica napus* L. (Bosac et al., 1994; Stewart et al., 1996). It has also been documented that O_3 decreases seed weight and quantity, and reduces lipid, protein and soluble carbohydrate contents as well as the root and shoot growth of seedlings for seed produced under elevated O_3 (Bosac et al., 1998).

Inhibition of reproductive growth in plants exposed to O_3 is mediated largely by reduced physiological efficiency of injured foliage (Kozlowski and Pollardy, 1997). By inducing premature leaf abscission and lowering photosynthetic capacity of leaves, O_3 decreases the amount of carbohydrates and growth regulators available for reproductive growth.

Since atmospheric levels of both CO_2 and O_3 are rising simultaneously, there is a critical need to examine how forest tree reproduction will be affected under these interacting greenhouse gases. We hypothesized that CO_2 will have largely positive effects, while O_3 will have adverse effects on seed production, germination and seedling development. Furthermore, we hypothesized that the effect of these gases on plant reproductive capacity will largely offset one another when they occur simultaneously. To test our hypotheses, we examined the impact of elevated CO_2 and/or elevated O_3 on paper birch reproduction at the Aspen FACE experiment where the trees have been exposed to elevated levels of these two greenhouse gases during the growing seasons of their 9 year life history (Karnosky et al., 2003, 2005).

2. Materials and methods

2.1. Study site

This study was done at the Aspen FACE site in Rhinelander, Wisconsin, USA (45.6°N, 89.5°W), which was established in 1997 as the first open-air facility to examine the responses of forest trees to interacting CO_2 and O_3 (Dickson et al., 2000). The Aspen FACE facility is situated at the U.S. Forest Service, Northern Research Station, Harshaw Research Farm near Rhinelander, WI, USA (Karnosky et al., 1999, 2003, 2005). The average annual precipitation at the site is 31.9 inches and an average summer temperatures range from 60 to 86°F. The soil is a coarse loam and a detailed description can be found in Dickson et al. (2000). The experimental site consists of four rings each of control (ambient air; CO_2 concentrations of 360 ppm), elevated CO_2 (560 ppm), elevated O_3 (1.5 × ambient) and elevated CO_2 plus elevated O_3 conditions in triplicate rings of 30-meters diameter each (Dickson et al., 2000).

2.2. Planting material

Birch seeds from a local upper Great Lakes seed source (Houghton County, Michigan) were collected and germinated in greenhouse conditions in 1997. These birch seedlings were transplanted into the Aspen FACE rings in July 1997. The birch seedlings were alternately planted with plants of the same size of trembling aspen clone 216 (which is fairly tolerant to ozone) in the southwest quadrant of each ring.

2.3. Flower, fruit and seed production

Due to the difficulty in accessing the canopy to count the number of flowers and seed-bearing catkins, a non-linear scoring scale was used as described by Darbah et al. (2007). Data on flowering were collected by scoring the quantity of flowering on each tree in each treatment (score 0 = no flowers; score 1 = 1–10 flowers per tree; score 2 = 10–100 flowers per tree and score 3 = more than 100 flowers per tree) for both male and female flowers and seed-bearing catkins (Darbah et al., 2007). Seed-bearing catkins were collected from all the treatments after scoring. Length, diameter and mass of the catkins were noted and mass/100 seeds were computed.

2.4. Seed germination and seedling development

Seed germination tests were done by selecting 100 healthy looking seeds (seeds with no deformity or damage) and germinating them in a petri dish on moist filter paper in a growth chamber. Distilled water was used to moisten the filter paper and the petri dishes were covered to control fungal growth. Temperature in the growth chamber was kept at $22 \pm 2\,°C$ under a 16 h photoperiod ($150\,\mu mol\,m^{-2}\,s^{-1}$ PAR from a white fluorescent light bulb). Neither fertilizer nor any chemical was added at this stage. The study was replicated four times making 400 seeds per treatment for each of the three years of seeds (2004, 2005 and 2006). Seeds were observed for radicle emergence, and upon emergence, hypocotyl height and cotyledon lengths were measured.

Seedlings were transferred into styroblocks (BC/CFC 4, B.C. Products Mfg.) in a greenhouse after three weeks in the petri dishes. The styroblocks had 160 wells (2.8 cm in diameter and 13.3 cm deep). Soil used was a mixture of top soil, perlite and peat moss in the ratio of 1:1:1; lime, and super phosphate and miracle grow fertilizer were applied. The seedlings were placed in a fog system in the greenhouse (Trion 500 Atomizer Humidifier model

500, 120 V) for the first week after transplantation. Temperature in the greenhouse was kept at 23–25 °C under a 16 h photoperiod (200 μmol m^{-2} s^{-1} PAR). Seedlings were later transplanted into 9.5 cm (diameter) × 11.5 cm (depth) pots. Height measurements were taken weekly till plants were five months old. Biomass of seedlings was determined after harvesting and oven drying the roots and shoots at 70 °C for 48 h.

2.5. Experimental design and data analysis

The experimental design of the Aspen Face project is a full factorial randomized complete block design with three replicate rings of four treatments (Dickson et al., 2000; Karnosky et al., 2003). Percentage germination values were arc–sin transformed ($\sin^{-1}\sqrt{x}$) to agree with the assumptions of normality before analyzing them. Analysis of variance (ANOVA) was used to test for significant differences between treatments for each year and average values ± standard errors presented and different letters used to indicate significant differences. Repeated measures analysis of variance (MANOVA) was also used for all the three seed years. Tukey's test significant at $P < 0.05$ level (Sokal and Rolph, 1995) was used to determine significant differences between treatment pairs. The PROC GLM component of the SAS statistical software (by SAS Institute.) was used in carrying out these analyses.

3. Results

3.1. Flower, fruit and seed production

It is worth noting that, for each growing season analyzed separately, we found significant treatment differences between most of the variables studied, but when all the seasons are analyzed together there is no significant difference between treatments. This was due to the fact that the number of trees flowering, quantities of flowers produced and seed germination rates differed significantly ($P < 0.001$) between years. Different microclimatic conditions as well as different physiological conditions of the trees in the three different years might explain these differences in reproductive capacity in the three years. There were no seeds produced under elevated $CO_2 + O_3$ in 2004 and 2005.

We recorded an increase of 140% ($P = 0.02$) and 70% ($P = 0.04$) for 2006 and 2007, respectively, in the total number of trees that produced male flowers under elevated CO_2 (Fig. 1a) and an increase of 260% ($P < 0.001$) in 2006 and 100% ($P = 0.01$) in 2007, respectively, in the quantity of male flowers produced under elevated CO_2 (Fig. 1c) relative to control. Elevated O_3 increased the number of male flower producing trees significantly ($P = 0.03$) in 2006 and ($P = 0.04$) in 2007 (Fig. 1a) even though the average score per tree was unaffected (Fig. 1b) resulting in increased flower production per treatment with $P = 0.009$ in 2006 and $P = 0.01$ in 2007 (Fig. 1c). We observed that under elevated $CO_2 + O_3$, male flower production was unaffected in 2006 but increased in 2007 with $P < 0.001$ (Fig. 1c) in the quantity of flowers produced per treatment.

There was no treatment differences in catkin size ($P > 0.05$) despite the 10% increase in size under elevated CO_2 and 20% decrease under elevated O_3 and no observable change under elevated $CO_2 + O_3$ relative to control (Table 1). The mass of seeds/100 seeds was increased significantly by 10% ($P = 0.03$ in 2004) under elevated CO_2 compared to
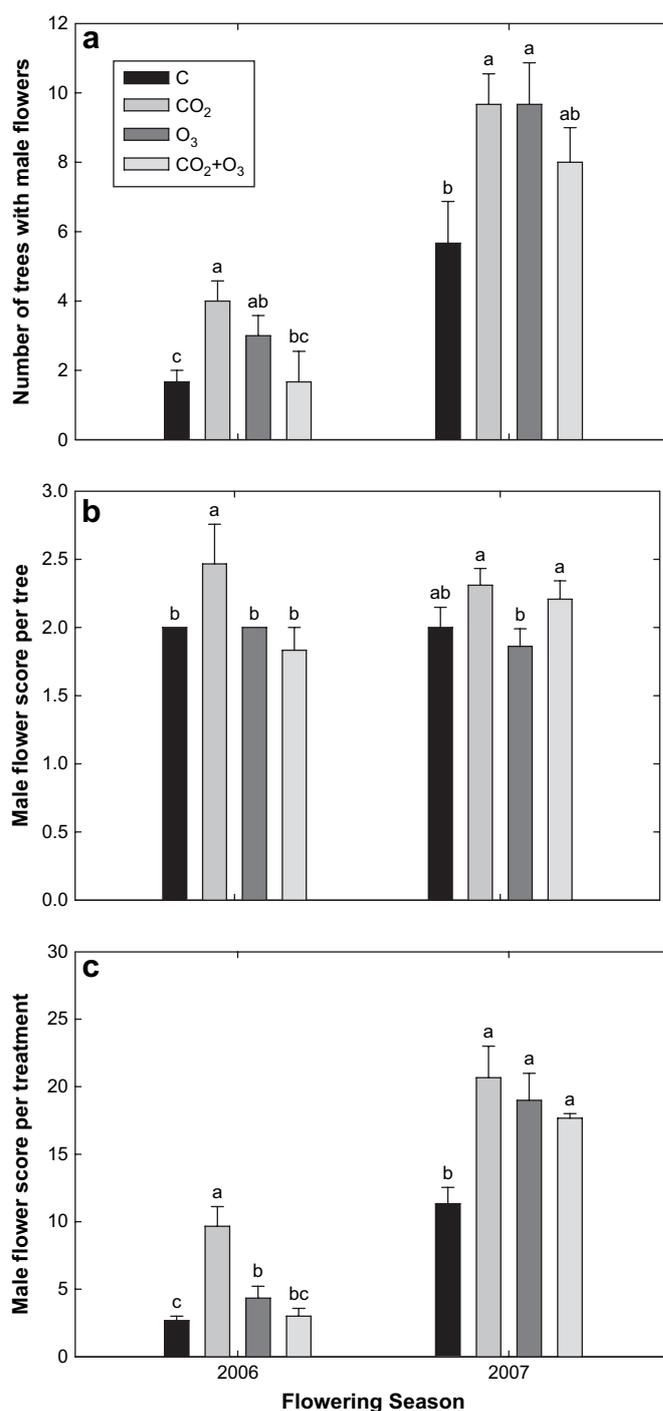

Fig. 1. Flower production in paper birch under elevated CO_2, O_3 and $CO_2 + O_3$ in two successive growing seasons observed at the Aspen FACE site in Rhinelander, WI, USA. Scoring for male flower buds were as follows: score 0 = no flowers; score 1 = 1–10 flowers per tree; score 2 = 10–100 flowers per tree and score 3 = more than 100 flowers per tree.

control, while elevated O_3 decreased seed mass/100 seeds significantly by 20% ($P = 0.03$) in 2004, 30% ($P = 0.04$) in 2005 and 20% ($P = 0.04$) in 2006 compared to control (Fig. 2). No seeds were found in 2004 and 2005 under elevated $CO_2 + O_3$, but we observed a 20% decrease ($P = 0.04$) in the seed mass of the seeds from this treatment in 2006.

Table 1
Seed and seedling characteristics (mean ± SE) of paper birch grown under ambient, elevated CO_2 (ambient +200 ppm) and elevated O_3 (1.5 × ambient) in 2004 at the Aspen Face site in Rhinelander, WI, USA

Parameter	Ambient	Elevated CO_2	Elevated O_3
Catkin length (mm)	33.04 ± 1.50	27.85 ± 0.87	30.80 ± 2.35
Catkin diameter (mm)	7.41 ± 0.20	8.46 ± 0.44	7.39 ± 0.38
Catkin mass (g)	0.64 ± 0.05	0.72 ± 0.07	0.59 ± 0.06
Seedling mortality (%)	2.75 ± 1.11	0.75 ± 0.48	2.50 ± 0.96
Root length (mm)	28.15 ± 5.56 b	44.68 ± 5.86 a	32.3 ± 8.31 ab
Shoot length (mm)	5.50 ± 0.77	6.65 ± 0.58	5.1 ± 0.44
Cotyledon length (mm)	2.64 ± 0.30	2.98 ± 0.27	2.5 ± 0.25
Seedling dry mass (mg)	16 ± 31 ab	27 ± 45 a	10 ± 20 b

No flowers or seeds were produced in the combined treatment in this year. Means are of four replicates ($n = 4$). Means within a row not followed by the same letter are significantly different at $P < 0.05$.

3.2. Seed germination and seedling development

For seeds developed under elevated CO_2, there were 70% ($P < 0.001$) and 110% ($P = 0.01$) increases in seed germination rates for the seed years 2004 and 2005 respectively, compared to control (Fig. 2). Seeds produced under elevated CO_2 and collected from the 2006 growing season did not differ significantly despite the 20% increase recorded relative to control. For seeds developed under elevated O_3, however, there were significant decreases of 70% ($P < 0.001$) in 2004, 60% ($P = 0.01$) in 2005 and 50% ($P < 0.001$) in 2006 in seed germination compared to controls. Seeds collected under elevated $CO_2 + O_3$ in 2006 had a 20% decrease in their germination rate compared to controls (Fig. 3). There were no treatment differences between germination rate of seeds from the control treatment and those from elevated $CO_2 + O_3$ despite the 20% reduction in germination rate under elevated $CO_2 + O_3$.

Even though there was no significant treatment difference ($P > 0.05$) in seedling survival, seedlings germinated from
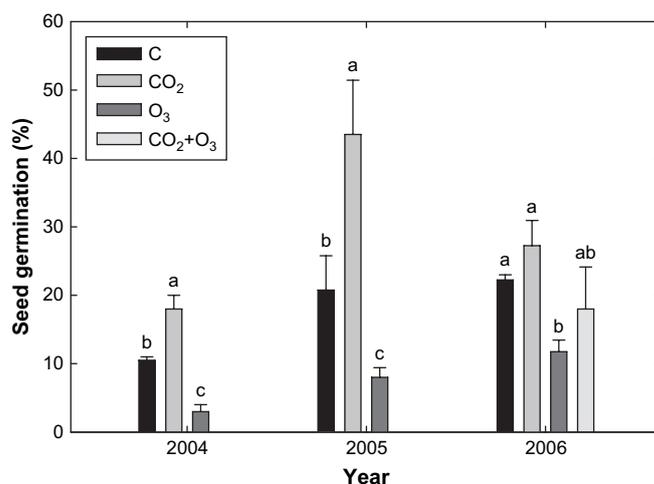

Fig. 3. Effects of elevated CO_2, O_3 and $CO_2 + O_3$ on seed germination success rate of paper birch seeds collected from the Aspen FACE site in Rhinelander, WI in 2004, 2005 and 2006 growing seasons No seed was formed in the $CO_2 + O_3$ treatment in 2004 and 2005.

seeds developed under elevated CO_2 had decreased mortality rates compared to those from elevated O_3 (Table 1). Despite the 20% increase in shoot length observed under elevated CO_2 there were not significant treatment differences recorded ($P > 0.05$) in either treatment. Root length was significantly increased by 60% ($P = 0.03$) under elevated CO_2, while elevated O_3 had no significant effect (Table 1). Seedlings from seeds produced under elevated CO_2 were consistently larger in size throughout the first five months while the opposite was true in the case of seedlings from elevated O_3 relative to control seedlings (Fig. 4). Seedlings from the elevated CO_2 treatment had significantly higher dry mass ($P = 0.03$) at the end of five months, on the other hand, seedlings from O_3 treatment did not differ significantly in their dry mass despite the 40% decrease in their dry mass.
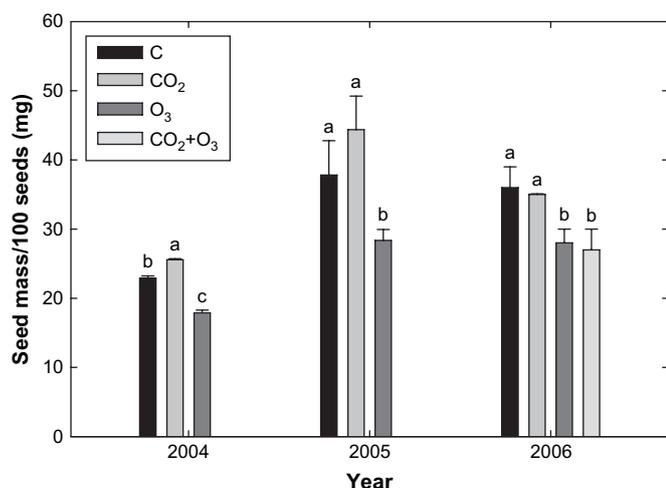

Fig. 2. Mass per 100 paper birch seeds produced under elevated CO_2, O_3 and $CO_2 + O_3$ in 2004, 2005 and 2006 growing seasons at the Aspen FACE site at Rhinelander, WI, USA. No seed was produced in the combined treatment in 2004 and 2005.
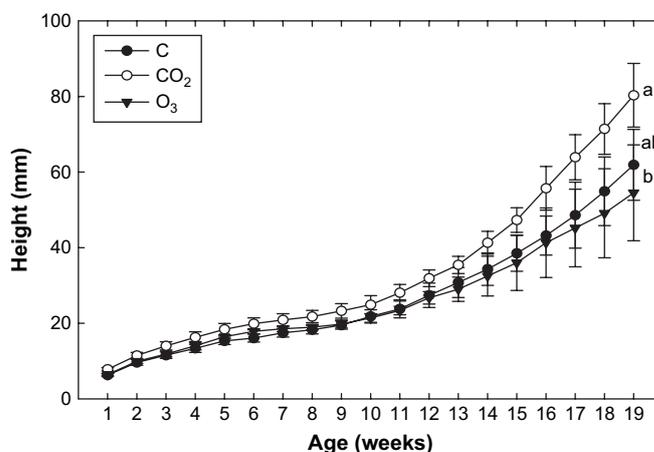

Fig. 4. Height growth for birch seedlings germinated under ambient conditions from seeds produced under elevated CO_2 and elevated O_3 at the Aspen FACE site in Rhinelander, WI in 2005. No seed was produced from the trees in the combined treatment in 2005.

4. Discussion

4.1. Flowering

Current knowledge on the impacts of elevated CO_2 and/or O_3 on forest tree reproductive and regenerative capacity is very limited compared to agricultural crops (Hussain et al., 2001). With atmospheric CO_2 and O_3 rising simultaneously, forest ecosystems will be exposed to these co-occurring greenhouse gases. The increases in the number of trees and in the quantity of male flowers produced under elevated CO_2 (Fig. 1) implies that more birch pollen will be produced. As birch pollen is an important inducer of allergic reactions (Pauli et al., 1996; Menz et al., 1996), our results suggest that birch pollen induced allergic reactions will be on the increase as CO_2 concentrations increase in the future. These results support the findings of Curtis et al. (1994, 1996), Johnson and Lincoln (2000), Edwards et al. (2001), Jablonski et al. (2002), Bunce (2005) and Ladeau and Clark (2006a,b) that elevated CO_2 increases reproductive potential through increased pollen production which, in turn, increases the probability of pollination, and hence, fertilization and viable seed formation. These results also support the hypothesis proposed by Herms and Mattson (1992) that birch trees under adequate carbohydrate status tend to favor male flower production.

We further found that O_3 also increased male flower production and these results are consistent with the findings of Chappelka, 2002 who reported of increased flowering in *Rubus cuneifolius* and Zvereva and Kozlov (2004) who documented enhanced sexual reproduction under pollution impact in dwarf shrubs. It has been suggested that plants increase carbon allocation to sexual reproduction under stressful conditions as an adaptive feature to ensure reproductive fitness of these genotypes (Saikkonen et al., 1998). This may also explain the increase in flowering under elevated CO_2 and O_3.

Under the elevated $CO_2 + O_3$ treatment we observed no change in the quantity of flowers produced in 2006 but an increase in 2007. Since our study had rather limited flowering and reproductive success in the combined treatment, we need additional data from subsequent seed years to better predict the nature of these responses for the interacting elevated CO_2 and O_3. It has been reported that elevated CO_2 ameliorates the effects of elevated O_3 for some physiological processes (example gas exchange; Volin et al., 1998; Karnosky et al., 2003) but more investigation is needed to determine if this is true for flower production.

4.2. Seed production

We found a significant increase in birch seed mass under elevated CO_2 compared to control in the 2004 and an increase of 20% in 2005 growing seasons (Fig. 2), which is in agreement with the observations made by Hussain et al. (2001), Jablonski et al. (2002) and Wang (2005). According to Hussain et al. (2001), the increase in the mass of seeds produced under elevated CO_2 was attributed to increased amounts of carbohydrate, lipids and proteins. They suggested that the increase in

these components of seed can have a profound effect on seed germination and seedling growth, as they provide stored resources for the germinating seedling. The extremely dry year, with a drought severity index of -3 to -3.9 according to the National Oceanic & Atmospheric Administration (NOAA) regional weather report for Rhinelander in 2006 (NOAA, 2006) may explain why there was no increase in seed mass under elevated CO_2 in 2006: birch trees under elevated CO_2 were larger and needed more water and therefore possibly suffered more from water stress than did the trees in control conditions.

The consistent significant decrease in seed mass in all three seed years observed under elevated O_3 (Fig. 2) supports the observations made by Bosac et al. (1998) who reported that elevated O_3 decreased seed weight and quantity. Their biochemical analysis on the seeds indicated that elevated O_3 had decreased lipid, protein and soluble carbohydrate contents in the seeds of oilseed rape (*Brassica napus*). Elevated O_3 is also known to reduce pollen germination which, in turn, can reduce the number of fertilized ovules which ultimately results in decreased seed mass (Black et al., 2000).

Seeds from elevated $CO_2 + O_3$ collected in 2006 had significantly reduced seed mass. There were no seeds produced under elevated $CO_2 + O_3$ in 2004 and 2005. In the 2006 seeds, no ameliorative effects of elevated CO_2 on O_3 were observed as the seed mass of seeds collected from O_3 and $CO_2 + O_3$ were similar in weight (elevated $O_3 = 28 \pm 2$ mg and $CO_2 + O_3 = 27 \pm 3$ mg, while control was 36 ± 3 mg). The first gene expression studies conducted following long-term exposure of trees to elevated $CO_2 + O_3$ reported of differential expression of genes that were not up-regulated with individual gas treatment (Gupta et al., 2005). This may be an indication that although elevated CO_2 may ameliorate the effects of elevated O_3 in some physiological processes such as gas exchange (Volin et al., 1998; Karnosky et al., 2003) it may not be so in all processes.

4.3. Germination

In this study, the germination period for all the four treatments (control, elevated CO_2, elevated O_3 and elevated $CO_2 + O_3$) ranged from 5 to 14 days and did not differ significantly between treatments. The higher germination rate of seeds collected from elevated CO_2 in both 2004 and 2005 is in agreement with the study conducted by Hussain et al. (2001) and Edwards et al. (2001). This increase in germination rate is possibly due to increased seed mass as a result of increased storage lipid, carbohydrate and protein content (Hussain et al., 2001). According to Tillman-Sutela et al. (1996), lipids in the seed provide metabolic energy for maturation of the embryo during seed development. Also, storage lipids are broken down during germination and converted to sugars and transported to cotyledons and axis for dry mass maintenance (Stone and Gifford, 1999). This might also explain why our germination rate was low for seeds collected under elevated CO_2 in 2006 during the high temperature and drought stress as Bosac et al. (1993) who reported that environmental stress can affect reproductive potential of plants.

Under elevated O_3, the observed decrease in seed germination was possibly due to decreased lipid content and hence, decreased metabolic energy as reported by Bosac et al. (1998) who found decreased seed quality in oilseed rape after exposure to O_3. Similarly, Marquez-Millano (1989) reported that germination success rate in *P. taeda* decreased in proportion to the loss of fatty acid content with seed aging. Increased abortion of seed buds was observed in oilseed rape (Bosac et al., 1994) and Brassica species (Stewart et al., 1996) exposed to elevated O_3. Also, reduced pollen germination and growth leading to decreased fertilization and fruit set was reported in oilseed rape exposed to elevated O_3 (Bosac et al., 1993).

There was no significant difference in seed germination rates under elevated $CO_2 + O_3$ despite the observed decrease in seed mass (20%) and germination rate (20%) in 2006 compared to control. It is possible that the positive effects of elevated CO_2 on storage lipid content ameliorated the negative effects of elevated O_3.

4.4. Seedling quality

For seeds produced under elevated CO_2, seedling vigor was higher than for seeds produced under control conditions, as seedlings were consistently larger throughout the first five months of the experiment (Fig. 4). Root length of seedlings from seed produced under elevated CO_2 was increased compared to seedlings from controls, enhancing the plants' ability to obtain water and nutrients (Table 1). This increased vigor of seedlings from seed produced under elevated CO_2 might be due to the higher amounts of stored carbohydrates, proteins and lipids that the seedling depends on initially for growth (Hussain et al., 2001) until its root system and photosynthetic apparatus are fully developed. This also supports the prediction made by Hussain et al. (2001) that seeds produced under CO_2-enriched environments may have fundamental changes in their viability, chemistry and germination that affect forest reproduction success.

The observed decrease in vigor of seedlings of seed produced under elevated O_3 in our study supports the observations made by Bosac et al. (1998) in oilseed rape that there is reduced seedling vigor in seeds produced under elevated O_3 conditions.

We also observed that seedlings from elevated CO_2 seeds had higher moisture contents, 74% compared to 60% in control and 43% in elevated O_3. This low moisture content or high proportion of biomass in plant mass in seedlings from elevated O_3 sources could be due to increased cell wall thickness, lignin and extractives in the elevated O_3 (seedlings), as reported by Anderson (2003).

Since we had very little seed produced under the interacting gases, we were not able to adequately characterize the seed and seedling characteristics for elevated $CO_2 + O_3$ treatment and this remains an important research priority for our work.

5. Conclusion

Our study documents the fact that both elevated CO_2 and elevated O_3 can impact the reproductive fitness of forest trees albiety in very different ways. Enhanced male flower production and increased seed weight, seed germination success rate, and seedling vigor was seen for seeds produced from trees exposed for their whole life history to elevated CO_2. Elevated O_3 similarly increased flower production but decreased the weight and germination success rate of seed from trees grown under this pollutant. Under interacting CO_2 and O_3, elevated CO_2 ameliorated the negative effects of elevated O_3 on flowering and seed germination, but seed mass was still reduced These findings suggest that reproductive and regenerative capacity of birch stands in the future could be altered by rising atmospheric CO_2 and O_3.

Since sexual reproductive development is an important stage in the life cycle of plants, any change in the processes involved might have significant implications for the productivity of the plants and their survival (Black et al., 2000). Understanding the potential interactive impacts of elevated CO_2 and O_3 on seed development and subsequent seedling performance of forest tree species is crucial for sustainable forest management in the presence of a changing global climate. Seedling responses play a key role in tree regeneration and succession, as germination and initial seedling growth set the pattern for future growth (Miao, 1995).

Acknowledgements

References

Anderson, C.P., 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytologist 157, 213–228.

Black, V.J., Black, C.R., Roberts, J.A., Stewart, C.A., 2000. Impact of the ozone on the reproductive development of plants. New Phytologist 147, 421–447.

Bobbink, R., 1998. Impacts of tropospheric ozone on airborne nitrogenous pollutants on natural and semi-natural ecosystems: a commentary. New Phytologist 139, 11–23.

Bosac, C., Black, V.J., Black, C.R., Roberts, J.A., Lockwood, F., 1993. Impact of O_3 and SO_2 on reproductive development in oilseed rape (*Brassica napus* L.). I. Pollen germination and pollen tube growth. New Phytologist 124 (3), 439–446.

Bosac, C., Black, V.J., Black, C.R., Roberts, J.A., Lockwood, F., 1994. Impact of O_3 and SO_2 on reproductive development in oilseed rape (*Brassica napus* L.). II. Reproductive site losses. New Phytologist 126, 71–79.

Bosac, C., Black, V.J., Roberts, J.A., Black, C.R., 1998. Impact of ozone on seed yield and quality and seedling vigour in oilseed rape (*Brassica napus* L.). Journal of Plant Physiology 153, 127–134.

Bunce, J.A., 2005. Seed yield of soybeans with daytime or continuous elevation of carbon dioxide under field conditions. Photosynthetica 43, 435–438.

Chappelka, A.H., 2002. Reproductive development of blackberry (*Rubus cuneifolius*), as influenced by ozone. New Phytologist 155, 249–255.

Curtis, P.S., Snow, A.A., Miller, A.S., 1994. Genotype – specific effects of elevated CO_2 on fecundity in wild radish (*Raphanus raphanistum*). Oecologia 97, 100–105.

Curtis, P.S., Klus, D.J., Kalisz, S., Tonsor, S.J., 1996. Intraspecific variation in CO_2 responses in *Raphanus raphinistum* and *Plantago lanceolata*: assessing the potential for evolutionary change with rising atmospheric change. In: Korner, C., Bazzaz, F.A. (Eds.), Carbon Dioxide, Populations and Communities. Academic Press, NY, pp. 13–22.

Darbah, J.N.T., Kubiske, M.E., Nelson, N., Oksanen, E., Vaapavuori, E., Karnosky, D.F., 2007. Impacts of elevated atmospheric CO_2 and O_3 on paper birch (Betula papyrifera): reproductive fitness. The Scientific World Journal 7 (S1), 240–246.

Dickson, R.E., Lewin, K.F., Isebrands, J.G., Coleman, M.D., Heilman, W.E., Riemenschneider, D.E., Sober, J., Host, G.E., Zak, D.R., Hendrey, G.R., Pregitzer, K.S., Karnosky, D.F., 2000. Forest Atmosphere Carbon Transfer Storage-II (FACTS II) – The Aspen Free-air CO_2 and O_3 Enrichment (FACE) Project in an Overview. USDA Forest Service North Central Research Station. General Technical Report NC-214, 68.

Edwards, G.R., Clark, H., Newton, P.C.D., 2001. The effects of elevated CO_2 on seed production and seedling recruitment in a sheep grazed pasture. Oecologia 127, 383–394.

Fowler, D., Cape, J.N., Coyle, M., Flechard, C., Kuylenstierna, J., Hicks, K., Derwent, D., Johnson, C., Stevenson, D., 1999. The global exposure of forests to air pollutants. Water, Air, and Soil. Environmental Pollution 116, 5–32.

Garbatt, K., Bazzaz, F.A., 1984. The effects of elevated CO_2 on plants. Lower, fruits and seed production and absorption. New Phytologist 98, 433–446.

Gupta, P., Duplessis, S., White, H., Karnosky, D.F., Martin, F., Podila, K., 2005. Gene expression patterns of trembling aspen trees following long-term exposure to interacting elevated CO_2 and tropospheric O_3. New Phytologist 167, 129–142.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Quarterly Review of Biology 67 (3), 283–335.

Hussain, M., Kubiske, M.E., Conner, K.F., 2001. Germination of CO_2 enriched *Pinus taeda* L. seeds and subsequent seedling growth responses to CO_2 enrichment. Functional Ecology 15, 1–7.

Idso, S.B., Kimball, B.A., 1997. Effects of long term atmospheric CO_2 enrichment on the growth and fruit production of sour orange trees. Global Change Biology 3, 89–96.

IPCC, 2007. Climate Change 2007. Working Group I Report: The Physical Basis of Climate Change. http://ipcc-wg1.ucar.edu/wg1/wg1-report.html.

Jablonski, L.M., Wang, X., Curtis, P.S., 2002. Plant reproduction under elevated CO_2 conditions: a meta analysis of reports on 79 crop and wild species. New Phytologist 156, 9–26.

Johnson, S.L., Lincoln, D.E., 2000. Allocation responses to CO_2 enrichment and defoliation by a native annual plant *Heterotheca subaxillariis*. Global Change Biology 6, 767–778.

Karnosky, D.F., Mankovska, B., Percy, K., Dickson, R.E., Podila, G.K., Sober, J., Noormets, A., Hendrey, G., Coleman, M.D., Kubiske, M., Pregitzer, K.S., Isebrands, J.G., 1999. Effects of troposopheric O_3 on trembling aspen and interaction with CO_2: Results from O_3-gradient and a FACE experiment. Water, Air and Soil Pollution 116, 311–322.

Karnosky, D.F., Zak, D.R., Pregitzer, K.S., Awmack, C.S., Bockheim, J.G., Dickson, R.E., Hendrey, G.R., Host, G.E., King, J.S., Kopper, B.J., Kruger, E.L., Kubiske, M.E., Lindroth, R.L., Mattson, W.J., McDonald, E.P., Noormets, A., Oksanen, E., Parsons, W.F.J., Percy, K.E., Podila, G.K., Riemenschneider, D.E., Sharma, P., Thakur, R., Sober, A., Sober, J., Jones, W.S., Anttonen, S., Vapaavuori, E., Mankovska, B., Heilman, W., Isebrands, J.G., 2003. Low levels of tropospheric O_3 moderate responses of temperate hardwood forests to elevated CO_2: a synthesis of results from the Aspen FACE project. Functional Ecology 17, 289–304.

Karnosky, D.F., Pregitzer, K.S., Zak, D.R., Kubiske, M.E., Hendery, G.R., Weinstein, D., Nosal, M., Percy, K.E., 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant, Cell and Environment 28, 965–981.

Kozlowski, T.T., Pollardy, S.G., 1997. Growth Control in Woody Plants. Academic Press. 279-283.

Ladeau, S.L., Clark, J.S., 2006a. Elevated CO_2 and tree fecundity: the role of tree size, interannual variability, and population heterogeneity. Global Change Biology 12, 822–833.

Ladeau, S.L., Clark, J.S., 2006b. Pollen production by *Pinus taeda* growing in elevated atmospheric CO_2. Functional Ecology 20, 541–547.

Marquez-Millano, A., 1989. Changes in vigor, leachate conductivity, and fatty acids composition of seeds of *Pinus elliottii englem. Var Elliottii* and *Pinus taeda* L. following accelerated aging. Ph.D. Dissertation, Mississippi State Univ., USA.

Menz, G., Dolecek, C., Schönheit-Kenn, U., Ferreira, F., Moser, M., Schneider, T., Suter, M., Boltz-Nitulescu, G., Ebner, C., Kraft, D., Valenta, R., 1996. Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v. I, the major birch pollen allergen. Clinical Experimental Allergy 26 (1), 50–60.

Miao, S., 1995. Acorn mass and seedling growth in *Quercus rubrain* response to elevated CO_2. Journal of Vegetation Science 6, 697–700.

NOAA, 2006. National Weather Service. Drought Severity Index by division. http://www.cpc.noaa.gov/products/analysis_monitoring/regional_monitoring/palmer/2006/07-22-2006.gif.

Pauli, G., Oster, J.P., Deviller, P., Heiss, S., Bessot, J.C., Susani, M., Ferreira, F., Kraft, D., Valenta, R., 1996. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. Journal of Allergy Clinical Immunology 97 (5), 1100–1109.

Piva, R.J., 1996. Pulpwood Production in the Lake States, 1994, Research Note NC-368. USDA. Forest Service, North Central Research Station, Rhinelander, WI, USA.

Prentice, I.C., 2001. The carbon cycle and atmospheric carbon dioxide. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (Eds.), Climate Change 2001: The Scientific Basis. Cambridge University Press, Cambridge, UK, pp. 183–238.

Ryerson, T.B., Frainer, M., Holloway, J.S., Parrish, D.D., Huey, L.G., Sueper, D.T., Frost, G.J., Donnelly, S.G., Schauffler, S., Atlas, E.L., Kuster, W.C., Goldan, P.D., Hubler, G., Meagher, J.F., Fehsenfeld, F.C., 2001. Observations of ozone formation in plant plumes and implications for ozone control strategies. Science 272, 719–723.

Saikkonen, K., Koivunen, S., Vuorisalo, T., Mutikainen, P., 1998. Interactive effects of pollination and heavy metals on resource allocation in *Potentilla anserine*. Ecology 79, 1620–1629.

Sokal, R.R., Rolph, F.J., 1995. Biometry, 3rd ed. WH Freeman and Co., New York, NY.

Stewart, C.A., 1998. Impact of ozone on the reproductive biology of Brassica campestris L. and Plantago major, PhD Thesis, University of Loughborough, UK.

Stewart, C.A., Black, V.J., Black, C.R., Roberts, J.A., 1996. Direct effects of ozone on the reproductive development of *Brassica* species. Journal of Plant Physiology 148, 172–178.

Stone, S.L., Gifford, D.J., 1999. Structural and biochemical changes in loblolly pine (*Pinus taeda* L.) seeds during germination and early seedling growth. II. Storage triacylglycerols and carbohydrates. International Journal of Plant Science 160, 663–671.

Tillman-Sutela, E., Johansson, A., Kauppi, A., Laakso, P., Kallio, H., 1996. Lipid and their seasonal variation in the nuclear layers of Scot pine (*Pinus sylvestris*) seeds. Canadian Journal of Botany 74, 1416–1424.

Volin, J.C., Reich, P.B., Givnish, T.J., 1998. Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. New Phytologist 138, 315–325.

Wang, X., 2005. Reproduction and progeny of *Silen latifolia* (Caryophylaceae) as affected by atmospheric CO_2 concentrations. American Journal of Botany 92, 826–832.

Ward, J.K., Strain, B.R., 1997. Effects of low and elevated CO_2 partial pressure on growth and reproduction of *Arabidopsis thaliana* from different elevations. Plant, Cell and Environment 20, 254–260.

Ward, J.K., Strain, B.R., 1999. Elevated CO_2 studies, past, present and future. Tree Physiology 19, 211–220.

Zvereva, E.L., Kozlov, M.V., 2004. Facilitative effects of top-canopy plants on four dwarf shrub species in habitats severely disturbed by pollution. Journal of Ecology 92, 288–296.