Growth of five hybrid poplar genotypes exposed to interacting elevated CO$_2$ and O$_3$


Abstract: A wide variety of hybrid poplar clones are being introduced for intensive culture biomass production, but the potential clonal or genotypic response to increasing tropospheric carbon dioxide (CO$_2$), ozone (O$_3$), and their interactions are unknown. To study these effects, we exposed five different hybrid Populus clones to increased concentrations of CO$_2$, O$_3$, and CO$_2$ + O$_3$ in open-top chambers for one growing season and determined growth responses. Exposure to elevated CO$_2$ increased height growth, dry mass, and basal area; exposure to O$_3$ decreased all three of these growth responses. Exposure impact differed among the different plant parts (leaf, stem, and roots) and among the clones. These differences were associated with different growth strategies or carbon allocation patterns inherent in the different clones. The fastest growing clones had the greatest response to O$_3$ treatment. The addition of CO$_2$ to the O$_3$ exposure counteracted the negative impact of O$_3$ in all plant components except leaf mass (e.g., CO$_2$ + O$_3$ plant mass equaled control plant mass) in all of the clones. But correspondingly, added O$_3$ negated increased growth from CO$_2$. Genetic variation in response to atmospheric pollutants must be considered even in closely related genotypes found in Populus culture.

Résumé: Une variété grande de clones de peupliers hybrides sont introduits pour la production de biomasse en culture intensive mais leurs réactions clonale ou génotypique potentielles face à l'augmentation du dioxyde de carbone (CO$_2$) et de l'ozone (O$_3$) dans la troposphère ainsi qu'aux interactions entre CO$_2$ et O$_3$ sont inconnues. Dans le but d'étudier ces effets, nous avons exposé cinq clones différents de peupliers hybrides à des concentrations élevées de CO$_2$, O$_3$, et de CO$_2$ + O$_3$ dans des chambres à ciel ouvert pendant une saison de croissance et nous avons mesuré les effets sur leur croissance. L'exposition à une quantité élevée de CO$_2$ a augmenté la croissance en hauteur, en masse sèche et en surface terrière; l'exposition à une concentration élevée de O$_3$ a réduit tous ces paramètres de croissance. L'impact de l'exposition variait selon la partie de la plante (feuilles, tige et racines) et selon le clone. Ces différences étaient associées à différentes stratégies de croissance ou patrons d'allocation du carbone inhérents aux différents clones. Les clones qui croissaient le plus vite ont réagi le plus à une exposition à O$_3$. L'addition de CO$_2$ lors de l'exposition à O$_3$ a contrecarré l'impact négatif de O$_3$ dans toutes les parties de la plante excepté dans le cas de la masse des feuilles (ex., la masse des plants exposés au CO$_2$ + O$_3$ était égale à la masse des plants témoins) chez tous les clones. Mais à l'inverse, l'addition de O$_3$ éliminait l'augmentation de croissance provoquée par le CO$_2$. La variation génétique dans la réaction aux polluants atmosphériques doit être considérée même chez les génotypes étroitement reliés qu'on retrouve dans la culture du peuplier.

[Traduit par la rédaction]

Introduction

Tropospheric carbon dioxide (CO$_2$) and ozone (O$_3$) are the two atmospheric pollutants generally considered to have the greatest impact on plant growth. Plant response to elevated CO$_2$ will be widespread because worldwide atmospheric concentrations are fairly uniform (Bazzaz 1990; Wittwer 1990; Bowes 1993). Plant response to elevated O$_3$ will be more localized because atmospheric concentrations vary widely in space and time (Chameides et al. 1994; Hogsett et al. 1997). Elevated CO$_2$ has the potential to increase productivity 20–50% in many agricultural crops and forest trees (Kimball 1983; Cure and Acoc 1986; Eamus and Jarvis 1989; Wittwer 1990; Ceulemans and Mousseau 1994). The 25% increase in atmospheric CO$_2$ concentration within the last 150 years may have already significantly increased productivity of crop plants (Wittwer 1990) and trees (Eamus and Jarvis 1989; Ceulemans and Mousseau 1994).

Ozone is a potent atmospheric pollutant that causes widespread damage to plants. Peak diurnal background O$_3$ concentrations in pristine areas currently range from 20 to 40 nL$^{-1}$ during the growing season. Summer daytime values of 50–70 nL$^{-1}$ (seasonal 70–100 µL·L$^{-1}$·h, 50 nL·L$^{-1}$·x
12-h day × 120-day growing season) are common over much of the eastern and southeastern United States (Taylor 1994; Hogsett et al. 1997) and southeastern Canada (Fuentes and Dann 1994) and are increasing by about 1–2% per year. More pessimistic estimates based on regional (eastern United States, Europe, China, and Japan) nitrogen oxide (NO\textsubscript{x}) production indicate that O\textsubscript{3} concentrations may triple within the next 30–40 years (Charneides et al. 1994). Damage estimates based on current O\textsubscript{3} concentrations indicate billions of dollars in agriculture crop losses annually (Adams et al. 1989) and significant impacts on forest tree productivity (Pye 1988; Taylor et al. 1994). However, decreases in yield of forest trees from O\textsubscript{3} impacts are not well documented. Estimates for major regional forest ecosystems are highly variable but on average range from a 2 to 15% decrease in growth over the next 20 years (de Steiguer et al. 1990). Estimates for more sensitive species (trembled aspen, Populus tremuloides Michx.; black cherry, Prunus serotina Ehrh.) range from a 14 to 33% loss in yearly productivity over 50% of their range in years of high O\textsubscript{3} impact (e.g., 1988, 1995). We have found decreases in total dry mass as high as 45% in sensitive aspen clones after one 97-day growing season of episodic O\textsubscript{3} exposure (92 μL·L\textsuperscript{-1}·h\textsuperscript{-1}) and 39% in a square-wave exposure (52 μL·L\textsuperscript{-1}·h\textsuperscript{-1}) (Karnosky et al. 1996).

Response to atmospheric pollutants varies among species and genotypes within species. Current damage estimates are usually based on broad species classification such as northern hardwoods or southern pines (de Steiguer et al. 1990) or average species response based on seedling populations (Pye 1988). However, these estimates do not account for the potentially large impact on sensitive genotypes within a species. Sensitive genotypes have been identified in both agricultural crops and forest trees (Kozlowski and Constantiniou 1986; Wittwer 1990; Taylor 1994; Karnosky et al. 1996; Ballach 1997). Most genetically improved tree species (and many agricultural crops) are selected based largely on growth rate but also on disease and stress resistance (Adams et al. 1992; Stettler et al. 1996). Such selection criteria may inadvertently select for O\textsubscript{3} sensitivity as well.

Because elevated CO\textsubscript{2} exposure usually increases photosynthetic rates, decreases stomatal conductance, and increases resistance to other environmental stresses, it is generally believed that increasing atmospheric CO\textsubscript{2} concentrations will offset the detrimental effects of increasing O\textsubscript{3} concentrations (Allen 1990). However, results of recent studies on the interacting effects of CO\textsubscript{2} and O\textsubscript{3} are contradictory. Some studies with several different species show that exposure to elevated concentrations of CO\textsubscript{2} may counteract decreases in photosynthesis and growth caused by O\textsubscript{3} (McKee et al. 1995; Mortensen 1995; Violin et al. 1998). In contrast, other studies show that elevated CO\textsubscript{2} did not protect against O\textsubscript{3} (Balaguer et al. 1995; Barnes et al. 1995). These studies involved average responses of general plant populations and did not examine genotypic responses. However, there is a strong genotypic response to both CO\textsubscript{2} (Ceulemans et al. 1996) and O\textsubscript{3} exposure (Karnosky et al. 1996) in Populus. We found that added CO\textsubscript{2} did not ameliorate the detrimental effects of O\textsubscript{3} on photosynthetic parameters of aspen clones differing in sensitivity to O\textsubscript{3}. In fact, the O\textsubscript{3}-tolerant clone appeared more sensitive to O\textsubscript{3} (Kull et al. 1996).

Because hybrid poplar clones differing in genetic makeup are being widely planted in reforestation and intensive culture systems in Canada, the United States, and many other countries around the world (Palmer 1991; Zsuffa et al. 1996; Riemenschneider et al. 1997), it is important to gather information about clonal response to these interacting atmospheric pollutants. Tropospheric CO\textsubscript{2} and O\textsubscript{3} are probably already impacting growth in sensitive genotypes, and these impacts will become more severe in the near future as atmospheric concentrations increase. Because of the potential importance of this information, we conducted a study to examine growth responses to elevated CO\textsubscript{2} and (or) O\textsubscript{3} of five poplar hybrid clones that are widely planted in both Canada and the United States (Brown et al. 1996). Our objectives were to examine the impact of CO\textsubscript{2}, O\textsubscript{3}, and CO\textsubscript{2} plus O\textsubscript{3} on growth and carbon allocation of poplar hybrids that differed in growth rates and inherent carbon allocation patterns (early shoot or root growth favored). Our hypotheses were (i) the more rapidly growing hybrids would show the greatest response to both CO\textsubscript{2} and O\textsubscript{3} exposure, (ii) hybrids favoring leaf production and height growth would show most O\textsubscript{3} response in root growth, and (iii) increased concentrations of CO\textsubscript{2} would significantly decrease the negative impact of O\textsubscript{3} exposure.

**Materials and methods**

**Plant material**

The plant material consisted of five hybrid poplar clones selected for a range of growth rates and carbon allocation strategies. All of the clones are high-productivity hybrids tested for growth response in Canada and released for commercial production (Brown et al. 1996). Four clones (DN-33, DN-34, DN-70, and DN-74) are *Populus deltoides* Bartr. × *P. nigra* hybrids and one clone (NM-6) is a *P. nigra* × *Populus maximowiczii* A. Henry hybrid.

Dormant hardwood cuttings (about 5 cm long) were planted in 6.5-L pots (15 × 35 cm) filled with a peat–sand–vermiculite mix (2:1:1), fertilized with 3.5 g/L slow-release fertilizer (Osmocote, Sierra Chemical Corp., Milpitas, Calif, 17:6:10, plus minor elements, 9-month formulation) mixed throughout the potting medium. The pots were watered daily to run through with a trickle irrigation system.

**Plant treatments and harvest**

The experiment was conducted at Michigan Technological University’s Ford Forestry Center in Alberta, Mich., during the summer of 1995. The four experimental exposure treatments were (i) charcoal-filtered control, (ii) elevated CO\textsubscript{2} (150 μL·L\textsuperscript{-1} above ambient), (iii) elevated ozone (100 nL·L\textsuperscript{-1} above the charcoal-filtered background), and (iv) elevated CO\textsubscript{2} plus elevated O\textsubscript{3} (at the above concentrations). The treatments were applied in open-top chambers (3.1 m wide × 4.6 m tall) (Karnosky et al. 1996) modified with frustums and rain exclusion caps. There were two chambers per treatment (total eight chambers) and 10 plants per clone in each chamber. The cuttings were planted on June 7, 1995, and exposures were started immediately so that the entire seasonal growth was under treatment. The elevated CO\textsubscript{2} treatment was applied for 24 h per day during the entire exposure period (June 7 to August 31; 86 days), and the square-wave O\textsubscript{3} treatment was applied for 6 h per day, 5 days per week (total 60 days). The total seasonal O\textsubscript{3} exposure was 12 μL·L\textsuperscript{-1}·h for the charcoal-filtered (CF) treatment and 48 μL·L\textsuperscript{-1}·h for the O\textsubscript{3} treatment.

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Table 1. Results of analyses of variance for whole plots.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Source of variation</th>
<th>Whole-plot error</th>
<th>O₃ × CO₂</th>
<th>CO₂</th>
<th>O₃</th>
<th>O₃ × CO₂ clone</th>
<th>CO₂ × clone</th>
<th>O₃ × clone</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>F</td>
<td>MS</td>
<td>F</td>
<td>MS</td>
<td>F</td>
<td>MS</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>19 165</td>
<td>5.01**</td>
<td>14 813</td>
<td>0.80ns</td>
<td>98 861</td>
<td>5.32†</td>
<td>147 396</td>
<td>7.94*</td>
<td></td>
</tr>
<tr>
<td>Total dry mass</td>
<td>4115.3</td>
<td>8.38**</td>
<td>588.2</td>
<td>0.15ns</td>
<td>19 833.6</td>
<td>4.99†</td>
<td>30 150.1</td>
<td>7.59*</td>
<td></td>
</tr>
<tr>
<td>Basal area</td>
<td>11 340</td>
<td>9.63**</td>
<td>264</td>
<td>0.02ns</td>
<td>43 532</td>
<td>3.98ns</td>
<td>75 670</td>
<td>6.91†</td>
<td></td>
</tr>
<tr>
<td>Leaf dry mass</td>
<td>358.5</td>
<td>9.44**</td>
<td>83.1</td>
<td>0.24ns</td>
<td>571.3</td>
<td>1.65ns</td>
<td>2386.6</td>
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<td>Stem dry mass</td>
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<td>72.1</td>
<td>0.06ns</td>
<td>7060.6</td>
<td>5.77†</td>
<td>8016.8</td>
<td>6.55†</td>
<td></td>
</tr>
<tr>
<td>Root dry mass</td>
<td>66.71</td>
<td>7.59**</td>
<td>16.83</td>
<td>0.26ns</td>
<td>245.18</td>
<td>3.80ns</td>
<td>369.23</td>
<td>5.73†</td>
<td></td>
</tr>
<tr>
<td>Shoot dry mass</td>
<td>3014.3</td>
<td>9.39**</td>
<td>435.9</td>
<td>0.11ns</td>
<td>11 544.0</td>
<td>4.08ns</td>
<td>21 418.4</td>
<td>6.71†</td>
<td></td>
</tr>
<tr>
<td>Cutting dry mass</td>
<td>19.78</td>
<td>2.94*</td>
<td>6.51</td>
<td>0.34ns</td>
<td>297.35</td>
<td>15.43*</td>
<td>257.08</td>
<td>13.34*</td>
<td></td>
</tr>
<tr>
<td>Shoot/root ratio</td>
<td>106.8</td>
<td>8.70**</td>
<td>161.9</td>
<td>1.57ns</td>
<td>102.7</td>
<td>1.00ns</td>
<td>132.8</td>
<td>1.29ns</td>
<td></td>
</tr>
<tr>
<td>Leaf/mass ratio</td>
<td>0.0146</td>
<td>5.46**</td>
<td>0.00027</td>
<td>0.02ns</td>
<td>0.11703</td>
<td>8.26*</td>
<td>0.00032</td>
<td>0.02ns</td>
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<tr>
<td>Stem/mass ratio</td>
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<td>2.12†</td>
<td>0.06140</td>
<td>4.06ns</td>
<td>0.20068</td>
<td>13.26*</td>
<td>0.10193</td>
<td>6.73†</td>
<td></td>
</tr>
<tr>
<td>Root/mass ratio</td>
<td>0.0074</td>
<td>8.11**</td>
<td>0.01470</td>
<td>2.05ns</td>
<td>0.00858</td>
<td>1.20ns</td>
<td>0.01178</td>
<td>1.64ns</td>
<td></td>
</tr>
<tr>
<td>Shoot/mass ratio</td>
<td>0.0305</td>
<td>3.33*</td>
<td>0.06916</td>
<td>2.27ns</td>
<td>0.02043</td>
<td>0.36ns</td>
<td>0.00914</td>
<td>2.95ns</td>
<td></td>
</tr>
<tr>
<td>Cutting/mass ratio</td>
<td>0.0163</td>
<td>1.09ns</td>
<td>0.14860</td>
<td>11.44*</td>
<td>0.03940</td>
<td>3.03ns</td>
<td>0.16790</td>
<td>12.93*</td>
<td></td>
</tr>
</tbody>
</table>

Note: Whole-plot error terms derived from chambers within O₃ × CO₂ treatment combinations. ns, mean square not significant (p > 0.10).
*Mean square significant (p < 0.05).
**Mean square significant (p < 0.01).

Table 2. Results of analyses of variance for subplots assuming all fixed-effects model.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Source of variation</th>
<th>Subplot error</th>
<th>O₃ × CO₂ clone</th>
<th>CO₂ × clone</th>
<th>O₃ × clone</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>MS</td>
<td>F</td>
<td>MS</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>3821</td>
<td>1041</td>
<td>0.27ns</td>
<td>1552</td>
<td>0.41ns</td>
<td>4285</td>
</tr>
<tr>
<td>Total dry mass</td>
<td>4913</td>
<td>142.8</td>
<td>0.29ns</td>
<td>1156.6</td>
<td>2.35*</td>
<td>1371.1</td>
</tr>
<tr>
<td>Basal area</td>
<td>1177</td>
<td>609.0</td>
<td>0.52ns</td>
<td>1245</td>
<td>1.06ns</td>
<td>1903</td>
</tr>
<tr>
<td>Leaf dry mass</td>
<td>38.0</td>
<td>14.3</td>
<td>0.38ns</td>
<td>54.8</td>
<td>1.44ns</td>
<td>91.9</td>
</tr>
<tr>
<td>Stem dry mass</td>
<td>144.0</td>
<td>62.3</td>
<td>0.43ns</td>
<td>381.6</td>
<td>2.65*</td>
<td>415.3</td>
</tr>
<tr>
<td>Root dry mass</td>
<td>8.78</td>
<td>6.91</td>
<td>0.79ns</td>
<td>17.1</td>
<td>1.94ns</td>
<td>19.3</td>
</tr>
<tr>
<td>Shoot dry mass</td>
<td>315.0</td>
<td>124.5</td>
<td>0.40ns</td>
<td>694.9</td>
<td>2.21†</td>
<td>888.5</td>
</tr>
<tr>
<td>Cutting dry mass</td>
<td>6.73</td>
<td>4.43</td>
<td>0.66ns</td>
<td>15.02</td>
<td>2.23†</td>
<td>11.75</td>
</tr>
<tr>
<td>Shoot/root ratio</td>
<td>12.3</td>
<td>36.5</td>
<td>2.98*</td>
<td>13.0</td>
<td>1.06ns</td>
<td>39.4</td>
</tr>
<tr>
<td>Leaf/mass ratio</td>
<td>0.00268</td>
<td>0.00150</td>
<td>0.56ns</td>
<td>0.00077</td>
<td>0.29ns</td>
<td>0.00427</td>
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<td>Stem/mass ratio</td>
<td>0.00728</td>
<td>0.00661</td>
<td>0.91ns</td>
<td>0.00334</td>
<td>0.56ns</td>
<td>0.01037</td>
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<tr>
<td>Root/mass ratio</td>
<td>0.00991</td>
<td>0.00116</td>
<td>1.27ns</td>
<td>0.00044</td>
<td>0.48ns</td>
<td>0.00109</td>
</tr>
<tr>
<td>Shoot/mass ratio</td>
<td>0.00949</td>
<td>0.00944</td>
<td>0.89ns</td>
<td>0.00682</td>
<td>0.72ns</td>
<td>0.01161</td>
</tr>
<tr>
<td>Cutting/mass ratio</td>
<td>0.01194</td>
<td>0.00763</td>
<td>0.64ns</td>
<td>0.00954</td>
<td>0.80ns</td>
<td>0.01850</td>
</tr>
</tbody>
</table>

Note: Subplot error terms derive from trees within clones within chambers within O₃ × CO₂ treatment combinations. ns, mean square not significant (p > 0.10).
*Mean square significant (p < 0.05).
**Mean square significant (p < 0.01).

Plants were harvested on September 6, 1995 (91 days from planting). During harvest, the height and diameter of each shoot on the cutting were measured (summed for each plant), and leaves, stems, cuttings, and roots were separated for each plant, dried at 70°C, and weighed.

Statistical analysis

Data were subjected to analyses of variance (ANOVA) according to a mixed-effect split-plot model. Whole-plot effects due to O₃, CO₂, and an O₃ × CO₂ interaction were assumed fixed and tested against a random whole-plot error (chambers within O₃ × CO₂ combinations) (Table 1). Subplot effects due to clone and the O₃ × clone, CO₂ × clone, and O₃ × CO₂ × clone interactions were also assumed fixed and tested against a random subplot error (clones × chambers within O₃ × CO₂ combinations) (Table 2). Unbalanced replication at the subplot level required complete least squares ANOVA and the use of least squares mean estimates for comparisons among treatment combinations. We did not adjust variance estimates or invoke special procedures to protect against type I error when making multiple comparisons, mostly because the validity of such procedures is unverified for unbalanced data sets (Steel and Torrie 1980). Some protection against type I error can be achieved by using simple least significant difference
Table 3. Relative response of six growth parameters of five Populus hybrid clones to elevated CO₂ and (or) O₃ exposure.

<table>
<thead>
<tr>
<th>Clones</th>
<th>Height</th>
<th>Total dry mass</th>
<th>Basal area</th>
<th>Leaf dry mass</th>
<th>Stem dry mass</th>
<th>Root dry mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>+32.6</td>
<td>-16.2</td>
<td>+20.2</td>
<td>-18.6</td>
<td>-5.8</td>
<td>-22.8</td>
</tr>
<tr>
<td>DN-70</td>
<td>+5.0</td>
<td>+34.0</td>
<td>+41.0</td>
<td>+18.6</td>
<td>+42.2</td>
<td>+62.5</td>
</tr>
<tr>
<td>DN-34</td>
<td>+13.1</td>
<td>+28.6</td>
<td>+27.2</td>
<td>+16.7</td>
<td>+37.9</td>
<td>+10.7</td>
</tr>
<tr>
<td>DN-74</td>
<td>+7.8</td>
<td>+34.2</td>
<td>+22.8</td>
<td>+5.5</td>
<td>+51.1</td>
<td>+45.6</td>
</tr>
<tr>
<td>NM-6</td>
<td>+22.3</td>
<td>+35.6</td>
<td>+36.1</td>
<td>+24.1</td>
<td>+52.6</td>
<td>+35.6</td>
</tr>
<tr>
<td>Mean</td>
<td>+16.2</td>
<td>+23.2</td>
<td>+29.4</td>
<td>+9.3</td>
<td>+35.6</td>
<td>+26.3</td>
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<td>O₃</td>
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<td>-46.7</td>
<td>-32.7</td>
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<td>-40.7</td>
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<td>-37.7</td>
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</tr>
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<td>-40.8</td>
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<tr>
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<td>-49.9</td>
<td>-41.8</td>
<td>-54.2</td>
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<td>-57.7</td>
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<tr>
<td>NM-6</td>
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<td>-41.9</td>
<td>-46.3</td>
<td>-57.5</td>
<td>-69.2</td>
</tr>
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<td>-38.1</td>
<td>-43.5</td>
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</tbody>
</table>

Note: Values are the percent change. A plus or minus sign indicates an increase or decrease in percent response compared with the charcoal-filtered control. See Figs. 1 and 2 for statistically significant differences among treatments.

comparisons to variables where an ANOVA F test is significant for one or more whole-plot treatments or their interactions (Steel and Torrie 1980).

Results

Growth responses

Whole-plant responses

Compared with control plants, exposure to elevated CO₂ increased height growth, total dry mass, and basal area; while elevated O₃ decreased all three of these growth parameters (Table 3, Fig. 1). Total dry mass and basal area changed the most in response to treatment while height was less responsive. Compared with the control plants, total dry mass and basal area of the treated plants increased 23 and 29% with CO₂ exposure and decreased 46 and 38% with O₃ exposure, respectively (Table 3). Height increased 16% with CO₂ exposure and decreased 28% with O₃ exposure. Response to the exposure treatments differed among the clones. In most cases, significant differences were found for all dependent variables measured (Tables 1 and 2). In some cases, however, pooled main effects were not significantly different (for example, basal area response to CO₂ treatment; Table 1) but were significantly different when the individual treatments were compared (Fig. 1). These apparently contradictory results arise because the F values in Table 1 come from pooled treatment comparisons (e.g., CO₂ and CO₂ + O₃ vs. control and O₃). Such pooled comparisons mask the strong and opposing growth responses to CO₂ and O₃ and the complex clonal interactions to elevated CO₂ and O₃.

In Fig. 1, the clones are placed in order of their relative growth response. The greatest response to treatment was associated with the fastest growing or most productive clones. Note that total dry mass production with O₃ exposure was similar for all clones, except DN-33 (Fig. 1B); while total mass in the CO₂ treatment was greater for NM-6 compared with all other clones. However, the percent increase in mass in response to CO₂, compared with the control treatment, was essentially the same in all clones (DN-70, 34%; NM-6, 36%) except in DN-33, which showed a slight but non-significant decrease in growth with the CO₂ exposure (Table 3). The decrease in mass in response to O₃ was greater in the more productive clones (e.g., NM-6, 50% and 31.3 g vs. DN-70, 41% and 16.5 g). In contrast to mass, the decrease in height in response to O₃ was similar (NM-6, 30%; DN-70, 30%) (Table 3), but the increase in height in response to CO₂ was greater in the more productive clones (NM-6, 22% vs. DN-70, 5%). Exposure of the different popular clones to CO₂ plus O₃ alleviated the detrimental response to elevated O₃. There were no significant differences between the control treatment and the CO₂ plus O₃ treatment in height, total dry mass, and basal area in any of the clones tested (Figs. 1A–1C). However, O₃ exposure negated the increase in growth from CO₂.

Leaf, stem, and root response

Growth of different plant parts (leaf, stem, and roots) also increased with CO₂ exposure and decreased with O₃ exposure (Table 3, Fig. 2). However, the relative response differed with the part in question and among the clones. These differences were associated with the different growth strategies or carbon allocation patterns inherent in the different clones. For example, DN-34 and NM-6 allocate considerable carbon to leaf growth, and exposure to elevated CO₂ increased leaf dry mass 17 and 24%, respectively (Table 3, Fig. 2A). Exposure to O₃ decreased leaf mass 41 and 46% in DN-34 and NM-6, respectively. In contrast, DN-74, allocating more carbon to stem and root growth, responded to elevated CO₂ with an increase in leaf mass of only 6% and responded to O₃ with a decrease of 54%. Conversely, exposure to elevated CO₂ increased root growth 11 and 36% in DN-34 and NM-6, respectively, while exposure to O₃ decreased root growth 63 and 69% (Table 3, Fig. 2C).
Fig. 1. Whole-plant response to exposures of elevated CO$_2$, O$_3$, and CO$_2$ + O$_3$: (A) total height growth, sum of all shoots on the cutting; (B) total plant dry mass; and (C) total basal area, sum of all shoots on the cutting. Treatments with the same letter are not significantly different at the 10% level based on the least significant difference test. Error bars are ± 1SE.

Response of the more root-oriented DN-70 showed an increase of 62% in root mass with CO$_2$ exposure and a decrease of 39% with O$_3$ exposure. These results show large clonal differences in carbon allocation within the plant in response to these environmental changes (Tables 1 and 2).

As with total plant growth, elevated CO$_2$ added to the elevated O$_3$ exposure largely counteracts the O$_3$ response, particularly in stems and roots (Figs. 2B and 2C). Average leaf mass was less, however, in the CO$_2$ plus O$_3$-treated plants compared with the controls but was significantly (statistically) smaller only in NM-6, the most productive clone (Fig. 2A).

Allometric responses
Carbon allocation patterns within the plant differ with clone, CO$_2$, and O$_3$ treatments. These different allometric responses are clearly shown when the different mass ratios are compared (Figs. 3 and 4). Shoot/root ratio is an allometric response that indicates important changes in carbon allocation within the plant and is often quite sensitive to changing environmental stresses. Ozone exposure increased the shoot/root ratio in DN-34 and NM-6 because root growth was impacted more than leaf and stem growth in these clones (Fig. 3A). Similarly, the shoot/root ratio decreased in DN-70 with CO$_2$ exposure because this clone allocates more available carbon to the root system. All other treatments had
Fig. 3. Allometric responses of leaves, stems, and roots to exposures of elevated CO$_2$, O$_3$, and CO$_2$ + O$_3$. (A) shoot/root ratio; (B) leaf/mass ratio (leaf mass divided by total plant dry mass); (C) stem/mass ratio. Significant differences are shown as in Fig. 1.

Fig. 4. Allometric responses of shoots, roots, and cuttings to exposures of elevated CO$_2$, O$_3$, and CO$_2$ + O$_3$. (A) root/mass ratio; (B) shoot/mass ratio (leaf and stem dry mass divided by total plant dry mass); (C) cutting/mass ratio. Significant differences are shown as in Fig. 1.

no effect on shoot/root ratios of the different clones compared with controls. Leaf mass in relation to total plant mass decreased with CO$_2$ exposure and CO$_2$ plus O$_3$ exposure, but O$_3$ exposure had little effect on the leaf mass ratios (Fig. 3B). This decrease in relative leaf mass was largely a CO$_2$ effect that was not counteracted by O$_3$ because the leaf mass ratio in the CO$_2$ plus O$_3$ treatment did not differ from that in CO$_2$ alone. Leaf mass in relation to whole-plant dry mass was smallest in DN-74 compared to the other four clones. In contrast to the leaf/mass ratio; stem/mass (Fig. 3C) and root/mass (Fig. 4A) ratios were significantly decreased by O$_3$ exposure in all clones except DN-74 (stem/mass ratio) and DN-70 and DN-74 (root/mass ratio). The decrease in root growth from O$_3$ exposure was particularly severe in the clones DN-34 and NM-6 (Fig. 4A).

Shoot/mass ratio decreased in the O$_3$-exposed plants but changed relatively little with the other treatments (Fig. 4B). This lack of shoot response in the CO$_2$ treatment reflects the average of leaf and stem response to treatment. For example, relative leaf mass decreased and stem mass increased with CO$_2$ exposure in clone NM-6 (Figs. 3B and 3C). While shoot/mass ratio decreased with O$_3$ exposure, cutting/mass ratio increased significantly with O$_3$ exposure in all clones.
Discussion

Based on the projected tropospheric concentrations of CO₂ and O₃ expected in 50–100 years (Bowes 1993; Taylor 1994), the CO₂ and O₃ exposures of this experiment were moderate. The CO₂ exposure was 150 µL·L⁻¹ above ambient or 510 µL·L⁻¹ for 86 days or slightly less than a full growing season (100–120 days in Alberta, Mich.), and the O₃ exposure summed for the treatment period was 48 µL·L⁻¹·h. The daily O₃ exposure of 100 nL·L⁻¹ 6 h per day is relatively high but not unusual for many areas in the eastern United States and southern Canada, where 70–100 nL·L⁻¹ daily maximum concentration is common and 150–190 nL·L⁻¹ for several hours during the day may occasionally occur (Fuentes and Dann 1994; Gillian and Turrill 1995). The summed experimental exposure of 48 µL·L⁻¹·h, if extended to a 100- to 120-day growing season, is well within the range of current seasonal ambient exposures (60–100 µL·L⁻¹·h) over much of the eastern United States and Canada (Fuentes and Dann 1994; Taylor 1994; Taylor et al. 1994; Hogsett et al. 1997). In addition, based on the current rate of increase of CO₂ and O₃, our experimental concentrations will equal ambient concentrations in about 50 years, if not sooner.

The square-wave O₃ exposure of 100 nL·L⁻¹ for 5 days per week may appear excessive. We have found, however, that such exposures provide several hours per day and 2 or 3 days per week for recovery in relatively low O₃ concentrations (20–30 nL·L⁻¹) but plant response to these square-wave exposures is greater than that obtained with episodic exposures that provide a similar accumulated O₃ dose (Karnosky et al. 1996). The greater impact probably results from more days of 100 nL·L⁻¹ O₃ exposure in the square-wave compared with the episodic treatment. Chronic ozone response of plants is determined by daily maximum concentration, total accumulated dose, and method of exposure (Taylor et al. 1994). The CO₂ concentration of 150 µL·L⁻¹ above ambient or 510 µL·L⁻¹ was chosen because this concentration more closely represented projected atmospheric CO₂ concentrations expected within the next 40–50 years. This time frame and CO₂ concentration is more realistic for hybrid poplar response (5 or 6 rotations in 50 years) than the 700 µL·L⁻¹ CO₂ concentrations projected within 100–150 years.

Plant growth usually increases when plants are exposed to increasing CO₂ concentrations because photosynthetic rates increase (Eamus and Jarvis 1989; Bazzaz 1990; Bowes 1993; Ceulemans and Mousseau 1994; Gunderson and Wullschleger 1994), respiration rates decrease (Bunce 1994; Wullschleger et al. 1994), and other stress effects may be alleviated (Cure and Acock 1986; Allen 1990; Wittwer 1990). Ozone exposure, in contrast, decreases photosynthetic rates, increases respiration rates, increases leaf senescence and leaf loss, and therefore, decreases plant growth and productivity (Pye 1988; Darrall 1989; Taylor 1994; Taylor et al. 1994; Coleman et al. 1995a; Karnosky et al. 1996). Both CO₂ and O₃ responses may be modified by cultural conditions, particularly nitrogen and water availability (Greitner et al. 1994; Pell et al. 1994; Curtis et al. 1995; Tschaplinski et al. 1995; Lloyd and Farquhar 1996). The poplar clones in this open-top experiment were grown in large pots with adequate fertilization and water to minimize stress and to provide rapid growing conditions so that response to CO₂ and O₃ exposure would not be confounded by other stresses. Poplar hybrids and Populus species in general are very responsive to environmental manipulation because of their inherent rapid growth rates and growth strategy designed to take advantage of favorable environmental conditions. Perhaps because of these characteristics, poplars are sensitive to increased CO₂ exposure and O₃ damage (Reich 1987; Laurence et al. 1994; Winner 1994; Karnosky et al. 1996).

The poplar clones in this experiment were no exception. Growth in all parameters measured (i.e., height; total dry mass; basal area; and leaf, stem, and root dry mass) increased with CO₂ exposure and decreased with O₃ exposure in all clones except DN-33 (Table 3, Figs. 1 and 2). An increase in plant mass is usually the result of an increase in photosynthetic rate or net carbon fixation rate. Reviews of the response of tree species to increasing CO₂ found that, depending on the experimental CO₂ concentration, average photosynthetic rates were 40–50% greater at the higher CO₂ concentrations than at ambient CO₂ concentrations (Gunderson and Wullschleger 1994; Curtis 1996). The response of all species was not positive and photosynthetic rates ranged from 40% less than that at ambient to three times ambient. Although increases in photosynthetic rates are not always directly related to increases in plant mass, a recent review found that average tree growth increased by about 40% (range 20–120%) with increased CO₂ concentration (Eamus and Jarvis 1989).

Reported responses to O₃ exposure are more variable than responses to CO₂. Dry mass responses ranged from a stimulation of 41% at low to moderate O₃ concentrations (1.5 times ambient) to decreases of 60–70% (Pye 1988). This large variability in response results from the extreme differences in experimental conditions and the use of species and genotypes that range widely in sensitivity. Differences in species response to O₃ have been frequently documented (Reich 1987; Mortensen and Skre 1990; Taylor et al. 1994). For example, studies with moderate levels of O₃ exposure (82 nL·L⁻¹, 7 h per day, 50 days or 29 µL·L⁻¹·h) showed that Betula pubescens Ehrh., was more sensitive than Betula verrucosa Ehrh., and the birches were more sensitive than alder (Alnus incana (L.) Moench). Total dry mass decreased 64 and 42% for birch and alder, respectively, with the 29 µL·L⁻¹·h treatment (Mortensen and Skre 1990). We found similar impacts of O₃ on our poplar clones as total dry mass decreased 46% (Table 3).

Plants respond to stress not only with changes in photosynthetic rates and growth rates but also with changes in carbon allocation within the plant. Carbon allocation within a plant depends on inherent growth strategy and response to varying environments (Chapin 1991; Lee and Jarvis 1995; Loehle 1996). Plant growth strategy differs widely among species. Species found in harsh environments commonly grow slowly and allocate most fixed carbon to root growth, storage, chemical defenses, or other functions that maximize
gains of limited resources and improve survival. Species found in rich environments often grow rapidly and allocate carbon to leaf and root development, organs that increase the capacity to acquire resources and increase growth rates, but rapidly growing species may be susceptible to stress. Within species and even within a genotype, carbon allocation may shift in response to environmental changes. A common response is the increased allocation of carbon to root growth when nitrogen or water is limited (Chapin 1991). Given the large number of species and hybrids involved in current Populus cultural and production studies (Stettler et al. 1996), it is not surprising that some genotypes vary widely in growth strategy and in response to environmental stress. Thus, carbon allocation within the plant and the resulting allometric patterns will also vary (Scarascia-Mugnozza et al. 1997). Early growth studies and tracer studies with photosynthetically fixed 14C clearly showed that during the first year of growth the hybrid "Tristis" allocated more carbon to root growth and developed larger root systems than Eugenie (DN-34), while Eugenie allocated more carbon to leaf development and height growth (Isebrands and Nelson 1983; Michael et al. 1988). Thus, Tristis growth strategy favored early root growth while Eugenie favored leaf production and height growth. We found similar differences in growth response in this current study with Populus hybrid clones that differed much less in parentage than Tristis and Eugenie. While DN-34 (Eugenie), DN-74, and NM-6 were all fast-growing hybrids, DN-74 had a smaller leaf/mass ratio than DN-34 and NM-6 (Fig. 3B), and a greater root/mass ratio (Fig. 4A). These genetically controlled growth responses are significant factors in the individual clonal response to elevated CO2, O3, and other environmental stresses.

The shoot/root ratio is probably the most common allometric parameter measured in studies of O3 or CO2 response of plants. Shoot/root ratios usually increase in plants exposed to O3 (Cooley and Manning 1987). This increase in shoot/root ratio is particularly common in indeterminate growing plants such as hybrid poplars (Matyssek et al. 1993; Woodbury et al. 1994). Both shoot and root growth usually decrease with O3 exposure. However, root growth is impacted more than shoot growth because lower leaves provide most of the photosynthate required for root growth and these lower or older leaves are the first leaf cohort damaged by O3 (Coleman et al. 1995a; 1996). Upper or recently mature leaves supply most of the photosynthate for new leaf and height growth and are least damaged by O3; thus, new leaf and shoot growth usually decrease less than root growth (Coleman et al. 1995b). The increase in shoot/root ratios of DN-34 and NM-6 (Fig. 3A) are actual allometric changes in carbon allocation and not a result of different growth rates (Gebauer et al. 1996) because total dry mass of all of the clones (except DN-33) were the same after ozone exposure.

The effect of increasing CO2 concentration on shoot/root ratios is much less certain. Shoot/root ratios may increase, decrease, or show no change in response to increasing CO2 depending on the species involved and experimental conditions (Eamus and Jarvis 1989; Ceulemans and Mousseau 1994; Tschaplinski et al. 1995; Gebauer et al. 1996; McConnaughay et al. 1996). Elevated CO2 often decreases the shoot/root ratio because increased photosynthetic rates provide excess carbohydrate that is preferentially utilized for root growth. We found relatively little effect of CO2 on shoot/root ratios compared with that found with O3 (Fig. 3A), although shoot/root ratio did decrease in DN-70, a more root-oriented hybrid. A similar lack of change in shoot/root ratio and other allometric responses with elevated CO2 exposure was found with several other hybrid poplar clones, although increases in total dry mass with CO2 exposure differed among these clones (Radoglou and Jarvis 1990; Bosac et al. 1995). The slowest growing clones often gave the greatest proportional increase in total dry mass with elevated CO2 compared with ambient (Radoglou and Jarvis 1990; Ceulemans et al. 1996). However, the fast-growing clones were always larger than the slow-growing clones in both ambient and elevated CO2. We found relatively little difference among the clones in percent dry mass increase with CO2 exposure compared with control plant dry mass (Table 3). However, this dry mass increase was not evenly distributed among the leaf, stem, and root fractions, indicating different allometric response to CO2 among the clones (Figs. 2 and 3).

Information on potential allometric changes in the different hybrids could be valuable because clones could be selected for different environmental conditions. For example, clones that favored root growth might be more drought tolerant or better able to utilize site nutrients. Such information would also be valuable if mixed hybrid plantings were desirable (Knowe et al. 1994).

Studies on the response of different species to CO2 exposure are more frequently reported than studies with O3 because of simplified experimental protocols and the concern about species and ecosystem response to increasing atmospheric CO2 concentrations (Bazzaz 1990). Most such studies report large differences in species response (Rochefort and Bazzaz 1992; Ackerly and Bazzaz 1995; Groninger et al. 1995; Tschaplinski et al. 1995; McConnaughay et al. 1996a), and in family or seed source response within species (Mebrahthly et al. 1993). Although recognized for some time, this within-species, genotypic response of forest trees to O3 or CO2 exposure has only recently been seriously considered (Taylor 1994; Taylor et al. 1994; Karnosky et al. 1996).

The range of genotypic response may be great. For example, the photosynthetic response to O3 exposure of 16 P. trichocarpa × P. deltoides F1 hybrids ranged from 5% of or equal to that found for the charcoal-filtered controls after 38 μL·L−1·h exposure (Hinckley 1996). Similar ranges in increased photosynthetic rates and growth rates may be expected in response to CO2 exposure (Eamus and Jarvis 1989; Radoglou and Jarvis 1990; Ceulemans and Mousseau 1994; Ceulemans et al. 1996). Given the wide genotypic range in O3 response found in sensitive tree species such as poplars, it is probable that considerable losses in productivity are already occurring at current ambient levels of O3 and that these losses will increase in the future.

In our study with hybrid poplars, the 48 μL·L−1·h O3 exposure decreased average dry mass of the five clones by 46% (Table 3). Similar responses to O3 were found for birch (Mortensen and Skre 1990) and aspen (Karnosky et al. 1996). The threshold for significant decreases in growth for sensitive genotypes will be considerably less than that found in average seedling populations or natural stands. Taylor (1994) reported that response of sensitive genotypes of
lobolly pine would begin around 30–40 μL·L⁻¹·h O₁ exposure and response of average seedling populations would be expected above 60–85 μL·L⁻¹·h. Growth response to our experimental O₁ exposure (48 μL·L⁻¹·h), when compared with current seasonal ambient O₁ exposures (60–100 μL·L⁻¹·h) found in much of the eastern United States and Canada indicate that sensitive genotypes are frequently severely impacted and whole populations are occasionally impacted by current O₁ concentrations, although decreases in growth may be less with ambient episodic exposures compared to our square-wave exposure. If tropospheric O₁ concentrations double in the near future as predicted (Chameides et al. 1994), actual yields of hybrid poplars and other fast-growing species in high O₁ impact areas will be much less than projected.

Most genetically improved tree species are selected based largely on growth rate, although disease and stress resistance are also important (Adams et al. 1992; Stetler et al. 1996). Selection based on growth rate alone may inadvertently select for O₁ sensitivity as well. In this study, the most rapidly growing hybrids had the greatest response to O₁ exposure. Although the percent productivity losses to O₁ exposure were similar (total dry mass decrease was 50.4% in NM-6 and 46.7% in DN-33), total dry mass losses were much greater in NM-6 than in DN-33 (e.g., 31.4 vs. 12.4 g). Based on our current knowledge of genetic control of O₁ resistance, selecting for O₁ resistance will be secondary to selecting for rapid growth rate. The most rapidly growing genotypes may do well in the future, however, because rapid growth may allow for rapid recovery after periodic O₁ stress and for greater productivity during years of low overall O₁ impact (Bazzaz and McConnaughay 1992; Wang et al. 1994). It should be possible in the near future to select for both rapid growth rate and resistance to various environmental stresses (e.g., O₁, drought, insects) (Robison and Raffa 1997) if clones are tested in both controlled environments (growth chambers, open-top chambers) and in the field. Multiyear field selection of clones planted in a number of different environments or sites along an O₁ gradient would be very useful. Genetic variation in response to atmospheric pollutants must be considered even in closely related genotypes found in Populus cultures because these pollutants will be a significant component of total environmental stress in the near future and may have significant silvicultural and management implications.

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References


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