Different Photosynthetic Responses of Black Cherry (*Prunus serotina*) with Different Sensitivities to Ambient Ozone Concentrations under Natural Conditions

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ABSTRACT

Two different sensitivity classes of black cherry (*Prunus serotina*) under the natural growing environmental conditions were assessed adjacent to Air Monitoring Station located at Horton research center in Giles County, Virginia, USA. Ambient ozone concentrations, leaf gas exchange, and visible foliar injury were measured on-site during the growing seasons of 2000, 2001, and 2002. Ambient ozone exposures were sufficient to induce typical foliar visible injury corresponding with the reduction in photosynthetic activities only in sensitive black cherry. There were positive correlations between increasing cumulative ozone concentration and percent reduction in maximum net photosynthetic rates (*PnMAX*) under saturating light conditions and in quantum yield for carbon reduction (*ΦCO₂*) of sensitive black cherry compared to tolerant black cherry. There was a negative correlation between chlorophyll content and percent leaf injury in sensitive black cherry. Furthermore, *PnMAX* was inversely related to percent leaf injury.

Key words: Photosynthetic activity, Ozone, Sensitivity, Black Cherry

I. Introduction

Ozone is considered as one of the most widespread and serious air pollutants affecting forest trees (Simini et al., 1992; Skelly, 2000). Ozone enters leaf through stomata, diffuses and reacts with the cellular membrane, and enters into metabolic processes (Reich, 1987). Therefore, ozone induced injury is closely related to physiological impairments with/without visible foliar injury. Samuelson (1994) reported the reduced photosynthetic rates and stomatal conductance accompanied with visible foliar injury when exposed to ambient ozone concentration.

Black cherry (*Prunus serotina*) is an economically and ecologically important in eastern forest of the United States (Rebbeck, 1996) and has been shown to be one of the most sensitive hardwoods to atmospheric ozone in terms of development of visible foliar injury.

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(Neaufeld et al., 1995). Palisade cells in the leaf mesophyll are particularly sensitive to ozone injury and the collapse of individual cells or groups of palisade cells produces visible symptoms on the leaf surface (Fredericksen, 1996). Older leaves are more severely affected with advancing symptoms of premature senescence and defoliation (Skelly et al., 1998).

Genetic variations in sensitivity to ozone have been documented for several broad-leaved forest tree species. Reference showed that visible foliar injury among individual black cherry seedlings differed considerably. For example, the degree of visible foliar injury depended on sizes and ages of black cherry trees, suggesting the existence of sensitive and tolerant genotypes (Kolb et al., 1997; Fredericksen, 1996).

It is challenging to assess direct and indirect effects of ozone under natural environmental conditions due to the diversity of environmental factors that affect exposure-response relationship (Schaub et al., 2005). The present study was designed to determine the physiological processes that may cause the differences in foliar injury and growth response between sensitive and tolerant black cherry when exposed to ambient ozone concentrations under natural environment conditions. The main objectives were 1) to compare visible foliar injury and leaf gas exchange of black cherry with different sensitivities and 2) to better understand physiological mechanisms that may explain different sensitivity in foliar injury and growth response to ozone between genotypes.

II. Materials and Method

2.1. Experimental site and design

This study was conducted for three consecutive years, 2000, 2001, and 2002 during the growing season from May to September. The site was selected in the vicinity of Air Quality Monitoring Station located at Horton Research center in Giles County, Virginia, USA. A TECO ozone analyzer was used to measure ambient ozone concentrations. Weather variables including wind speed, wind direction, solar radiation, temperature, relative humidity (RH), and rainfall were monitored at the station. Two groups of black cherry (Prunus Serotina Ehl.) with contrasting sensitivities were selected on site. The sensitivity was determined as symptomatic foliar injury, occurring as upper leaf flecking and dead tissue, during the previous summer. Three trees were selected to represent two sensitivity classes, sensitive and tolerant, respectively.

2.2. Gas Exchange

Gas exchange analysis was performed on the 3rd basal leaf every month from May to September each year. Gas exchange analysis was conducted with a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE). Net photosynthetic rate under saturating light conditions (PmMAX, µmol CO2 m−2 s−1) at a light intensity of 900 µmol quanta m−2 s−1 PAR and 350 ppm CO2) and stomatal conductance (gs, mol H2O m−2 s−1) were determined monthly from May to September on the 3rd leaf position each year. Assimilation-Irradiance response curves (A-I) were obtained by measuring assimilation at 350 ppm CO2 concentration and irradiance ranging from 0 to 1200 µmol m−2 s−1 PAR monthly from May to September during the growing season. From the initial slope of A-I curve, the apparent quantum efficiency for net CO2 assimilation (CO2) was determined. Assimilation - CO2 response curves were also generated at maximum light condition of 1100 µmol m−2 s−1 PAR and CO2 concentrations ranging from 0 to 1600 ppm.

2.3. Visible foliar injury

Visible injury was evaluated at two week intervals beginning in mid-May until the end of the season in mid-September each year. Leaf positions two through six in sensitive trees were examined. The percentage of visible injury of total leaf area was assessed on the upper leaf surface.

Leaf discs were used to determine total chlorophyll concentrations. Extraction was performed using pure DMSO solvent. Concentrations of chlorophyll a and b were determined spectrophotometrically at wavelengths of 663 and 645, respectively, according to Barnes et al. (1992) and related to the fresh weight of the leaves. Total chlorophyll concentrations were quantified and compared between sensitive and tolerant black cherry on the 3rd leaves during the growing season, June to August. In September from sensitive trees, the relationship between total chlorophyll concentrations and percentage leaf injury was evaluated.

2.4. Data analysis

Two branches per tree and three trees per sensitivity class (i.e., sensitive and tolerant) were chosen on site. Each tree was designated as an experimental unit and each branch represented a pseudoreplicate. Statistical
analysis of the data was performed with the Statistical Analysis System (SAS, Inc., NC, USA). Photosynthetic data as well as chlorophyll fluorescence data were analyzed by analysis of variance with ozone sensitivity as class variable. Statistical significance was designated at the $P \leq 0.05$ probability level by a single asterisk and the $P \leq 0.01$ level by two asterisks.

III. Results

3.1. Ozone exposure

Black cherry trees were exposed to ambient ozone for 7 hours (from 09:00 to 16:00 hrs). On average, the concentration of ambient ozone was 51, 51, and 53 ppb in 2000, 2001, and 2002, respectively. One-hour peak value of ambient ozone was higher in 2002 compared to those in 2000 and 2001 (Table 1). The highest one-hour peak in 2000 and 2001 were recorded in June at 90 and 95 ppb, respectively. The highest one-hour peak in 2002 was recorded in July at 121 ppb (Fig. 1). The seasonal SUM00 and SUM40 ozone values were higher in 2002 compared to 2000 and 2001 (Table 1, Fig. 2).

3.2. Physiological gas exchange

For three growing seasons of investigation, photosynthetic activities differed between sensitivity classes in black cherry. The ambient ozone concentrations were high enough to induce the reductions of photosynthetic activity in sensitive class compared to tolerant class. The substantial reductions of $P_{\text{MAX}}$ and $\Phi_{\text{CO}_2}$ in sensitive class were observed throughout the experimental seasons for the three years. Such reductions were associated with seasonal cumulative ambient ozone exposure (SUM00). For example, $R^2$ value for $P_{\text{MAX}}$ and $\Phi_{\text{CO}_2}$ were 0.80 and 0.82, respectively. The values of $P_{\text{MAX}}$ and $\Phi_{\text{CO}_2}$ for sensitive class increased at first.

### Table 1.

<table>
<thead>
<tr>
<th>Seasonal means of atmospheric ambient $O_3$ concentrations (ppb) and seasonal cumulative $O_3$ concentrations (ppm h) at Horon research center in Giles County, VA, for three consecutive years from 2000 to 2002. Values are means ± s.d.</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-h means</td>
<td>51±13</td>
<td>51±12</td>
<td>53±13</td>
</tr>
<tr>
<td>12-h means</td>
<td>51±12</td>
<td>51±11</td>
<td>53±13</td>
</tr>
<tr>
<td>Peak means</td>
<td>58±13</td>
<td>62±12</td>
<td>66±16</td>
</tr>
<tr>
<td>Seasonal peak</td>
<td>90</td>
<td>95</td>
<td>121</td>
</tr>
<tr>
<td>SUM40 (ppm h)</td>
<td>NA</td>
<td>67.46</td>
<td>75.77</td>
</tr>
<tr>
<td>SUM00 (ppm h)</td>
<td>80.47</td>
<td>81.38</td>
<td>85.02</td>
</tr>
</tbody>
</table>

*NA: not available
Table 2. Maximum net photosynthetic rate (PnMAX; µmol CO₂ m⁻² s⁻¹ at 900 µmol CO₂ m⁻² s⁻¹) stomatal conductance (gₛ; mol H₂O m⁻² s⁻¹), internal CO₂ concentration (Cᵢ, ppm) and apparent quantum yield for CO₂ assimilation (Φ₁) on the 3rd basal leaf of sensitive and tolerant black cherry during the growing season of 2000. Values are mean ± s.d. Asterisks indicate the significant differences between cultivars. * at P<0.05 and ** at P<0.01.

<table>
<thead>
<tr>
<th></th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>PnMAX</td>
<td>9.16±0.67</td>
<td>7.42±0.42**</td>
<td>11.27±0.94</td>
<td>10.85±1.30</td>
<td>8.93±0.25</td>
</tr>
<tr>
<td>gₛ</td>
<td>0.17±0.02</td>
<td>0.10±0.01**</td>
<td>0.30±0.02</td>
<td>0.26±0.04</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>Cᵢ</td>
<td>234±7.2</td>
<td>206±9.1**</td>
<td>259±4.2</td>
<td>254±5.1</td>
<td>256±6.1</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.30±0.002</td>
<td>0.46±0.003</td>
<td>–</td>
<td>–</td>
<td>0.07±0.001</td>
</tr>
</tbody>
</table>

− : data not available
June, Pn values for sensitive and tolerant black cherry were not different significantly under all light conditions. From July, however, Pn under light conditions higher than 150 µmol m$^{-2}$ s$^{-1}$ PAR reduced significantly in sensitive black cherry compared to tolerant black cherry.

Net CO$_2$ assimilation rates in response to various CO$_2$ concentrations were measured during the experimental season in 2002. A-CO$_2$ response curves were generated and compared between sensitive and tolerant black cherry (Fig. 5). In 2002, Pn increased as CO$_2$ concentrations increased (Fig. 5). In June, Pn, at lower CO$_2$ concentration than ambient conditions of 350 ppm, was not significantly different in both sensitive and tolerant black cherry. However, Pn, at higher CO$_2$ conditions than 350 ppm, was significantly reduced in sensitive black cherry compared to tolerant black cherry. Under the doubled ambient CO$_2$ conditions (i.e., > 800 ppm), the reduction of Pn in sensitive black cherry was 20% compared to that of tolerant black cherry. From July, Pn, at ambient CO$_2$ conditions of 350 ppm, was reduced in sensitive black cherry compared to tolerant black cherry. By August, at even lower CO$_2$ conditions, Pn was significantly reduced in sensitive black cherry compared to tolerant black cherry. In August, reductions of Pn in sensitive black cherry compared to tolerant black cherry were getting greater as CO$_2$ concentrations increased.

The values of $\Phi_{CE}$ for both sensitive and tolerant black cherry were not significantly different in June. However, from July, reductions of $\Phi_{CE}$ in sensitive black cherry compared to tolerant black cherry were significant. By August, reduction of $\Phi_{CE}$ in sensitive black cherry was more than 40% compared to tolerant black cherry (data not shown).

3.3. Visible foliar injury

From late July to early August, visible foliar injury
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Towards the end of growing season in September, most leaves showed visible injury in sensitive black cherry. The symptoms appeared as brown spots on the upper surface of leaves, which is typical foliar injury induced by ozone in black cherry. The estimated foliar injury was more severe in older leaves with 30 to 35% of total leaf area than in younger leaves with 0 to 5% (Fig. 6). This visible foliar injury was well correlated with photosynthetic activity and chlorophyll contents. $P_{\text{MAX}}$ was related inversely to percent visible foliar injury with an $R^2$ value of 0.83 in 2000 and 0.89 in 2001 (Fig. 7). Chlorophyll contents were also related inversely to percent visible foliar injury with an $R^2$ value of 0.95 (Fig. 8).

Fig. 5. Net assimilation ($P_n$; mol CO$_2$ m$^{-2}$ s$^{-1}$) - CO$_2$ concentrations (ppm) response curves (at 1100 µmol m$^{-2}$ s$^{-1}$ PAR) on the 3rd leaf of sensitive (closed circles) and tolerant (open circles) black cherry during the growing season of 2002. Bars represent ± one standard deviation of means and, where not apparent, are contained within symbols.

Fig. 6. Percent visible foliar injury of total leaf area estimated on the different leaf positions of sensitive black cherry in September 2001. Leaf position 10 (L10) is the youngest leaf estimated. Error bars represent standard deviations of the means.

Fig. 7. $P_{\text{MAX}}$ (maximum net photosynthetic rate, µmol CO$_2$ m$^{-2}$ s$^{-1}$ at saturated light condition of 900 µmol m$^{-2}$ s$^{-1}$) related to percent visible foliar injury of sensitive black cherry in consecutive 2 years, 2000 (solid line and closed circles) and 2001 (dotted line and open circles).
IV. Discussion

In our study, level of exposure to ambient ozone in terms of concentrations and duration was high enough to cause visible foliar injury and to disrupt physiological activity only in sensitive black cherry. Our results were similar to those reported by Schaub et al. (2005). The seasonal 7-h and 12-h ambient ozone concentrations averaged 40-50 ppb (Table 1) and episodic peaks reached 90 to 120 ppb (Fig. 1), which typically occurs during the summer in Mid-Atlantic States (Skelly, 2000 and Schaub, 2003).

Our results showed that visible foliar injury of sensitive black cherry resulted from greater stomatal conductance and consequently greater ozone uptake than those of tolerant black cherry (Table 2). High ozone uptake with greater stomatal conductance in sensitive black cherry seedlings has been linked to greater ozone injury than tolerant black cherry seedlings (Kouterick et al., 2000). Our results confirmed that foliar injury was correlated with stomatal conductance in black cherry. For example, Ferdinand et al. (2000) reported that stomatal density of sensitive black cherry seedlings was greater than that of tolerant ones because the sensitive seedlings had greater potential of stomatal conductance than tolerant ones. Even though ozone tolerant mature black cherry showed greater leaf area, stomatal density was still significantly greater for ozone sensitive black cherry.

The response to ozone is underestimated, however, if sensitivity emphasizes only the differences in stomatal conductance (Schaub et al., 2005). Not only stomatal density but other internal morphological leaf characteristic may play an important role in response to ozone of sensitive black cherry. Fredericksen et al. (1995) reported thinner leaves and a lower palisade/spongy mesophyll thickness ratio in sensitive black cherry than tolerant black cherry. According to Ferdinand et al. (2000), leaves of R-12, ozone-sensitive seedlings, displayed greater spongy mesophyll with greater intercellular air space and thinner palisade mesophyll than MO-7, ozone-tolerant seedlings. These morphological characteristics in sensitive black cherry would provide less mesophyll resistance to ozone ingress to the palisade mesophyll and may lead dead palisade mesophyll cells and visible foliar injury. Evans et al. (1996) also supported that certain leaf morphological characteristics such as stomatal density and intercellular air space may lead ozone ingress into leaves and internal diffusivity among ozone target cells, such as palisade mesophyll cells. Although direct morphological characteristics have not been measured, a clear linear relationship between chlorophyll contents and foliar injury were observed in sensitive black cherry with R² value of 0.95 in this study (Fig. 8). As chlorophyll contents decreased, greater visible foliar injury was observed.

Kouterick et al. (2000) reported that the differences in ozone uptake and visible foliar injury between families did not correspond with family differences in photosynthetic activity to ozone exposure. They observed that the relative ozone sensitive family R-12 had more visible foliar injury as well as higher Pn than relative ozone tolerant family MO-7 throughout the growing season. In addition, all measurements of Pn were 12 to 17 µmol CO₂ m⁻² s⁻¹ for sensitive family R-12 and 10 to 16 µmol CO₂ m⁻² s⁻¹ for tolerant family MO-7 under 600 µmol m⁻² s⁻¹ light conditions regardless to seasonal cumulative ozone concentration. However, they measured Pn of newly expanded young leaf throughout the growing season and cumulative effect of ozone was not considered. Such young leaves would have higher Pn and yet would not be severely affected by ozone. Our results clearly showed that reductions of Pn in sensitive black cherry compared to tolerant black cherry were correlated with seasonal cumulative ozone concentrations (Fig. 3).

Greater foliar injury in the older leaves was observed in sensitive black cherry at the end of growing season due to a longer duration of ozone exposure than younger leaves produced later in the growing season (Fig. 6). Older leaves also showed more severe injury in PnMAX than younger leaves in sensitive black cherry (data not shown). Visible foliar injury was well correlated to photosynthetic activity in this study. The per-
cent leaf injury of total leaf area was negatively related to Pmax in sensitive black cherry with R² value of 0.83 and 0.89 in 2000 and 2001, respectively (Fig. 7). Our results agree with other studies on other ozone sensitive trees and crops, which showed a clear inverse relationship between visible foliar injury and photosynthetic activity (Davis and Skelly, 1992; Flagler, 1994). Our results indicated that visible foliar injury was related to photosynthetic activity between black cherry families. Therefore, visible foliar injury could be used as a simple measure to study ozone impacts on black cherry in terms of genetic difference.

With limited access to biochemical mechanisms such as direct measurement of production and activation of RUBISCO, carboxylation efficiency was determined from the assimilation-CO₂ response curves during the growing season in 2002 and compared between sensitive and tolerant black cherry (Fig. 5). Carboxylation efficiency in sensitive black cherry was reduced in July with other photosynthetic activity. When carbon is not a limiting factor under saturating CO₂ conditions, however, the maximum rate of electron transport used in the regeneration of RUBP was reduced in sensitive black cherry at first.

This study demonstrated the different genetic responses of black cherry to ambient ozone concentrations under the natural environmental conditions. The genotypic differences in response to ozone have been clearly observed. Further researches in genetic variations of ozone sensitivity among black cherry should include examinations of biochemical analysis such as anti-oxidant production and RUBISCO activity levels which differ in sensitivity to foliar ozone injury.

REFERENCES


