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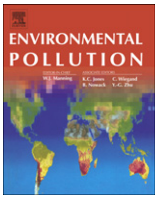

Leaf age affects the responses of foliar injury and gas exchange to tropospheric ozone in *Prunus serotina* seedlings

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Ozone effects on symptom development and leaf gas exchange interacted with leaf age and N-content on black cherry seedlings.

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ABSTRACT

We investigated the effect of leaf age on the response of net photosynthesis (A), stomatal conductance (g_{wv}), foliar injury, and leaf nitrogen concentration (N_L) to tropospheric ozone (O_3) on *Prunus serotina* seedlings grown in open-plots (AA) and open-top chambers, supplied with either carbon-filtered or non-filtered air. We found significant variation in A, g_{wv}, foliar injury, and N_L ($P < 0.05$) among O_3 treatments. Seedlings in AA showed the highest A and g_{wv} due to relatively low vapor pressure deficit (VPD). Older leaves showed significantly lower A, g_{wv}, N_L, and higher foliar injury ($P < 0.001$) than younger leaves. Leaf age affected the response of A, g_{wv}, and foliar injury to O_3. Both VPD and N_L had a strong influence on leaf gas exchange. Foliar O_3-induced injury appeared when cumulative O_3 uptake reached 8–12 mmol m^{-2}, depending on soil water availability. The mechanistic assessment of O_3-induced injury is a valuable approach for a biologically relevant O_3 risk assessment for forest trees.

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1. Introduction

Tropospheric ozone (O_3) has been, and is predicted to remain, the most harmful to vegetation of all regionally dispersed air pollutants (Hogsett et al., 1988; Davison and Barnes, 1998; Vingarzan, 2004; Karnosky et al., 2007). Effects of commonly observed atmospheric O_3 concentrations on plant functionning have been well-documented (Winner, 1994; Sandermann, 1996; Matyssek et al., 2008). Because O_3 diffuses into leaves together with carbon dioxide, stomatal regulation has been considered as an important factor in controlling O_3 sensitivity of plants (Reich, 1987; Becker et al., 1989; Taylor and Hanson, 1992; Matyssek and Sandermann, 2003; Paoletti and Grulke, 2005). After O_3 enters the intercellular space of stomata, O_3 dissociates quickly to form oxygen and peroxides (Laisk et al., 1989; Lange et al., 1989; Larcher, 1995). It is the peroxide that oxidizes protein and lipid components of cellular membranes and thereby disrupts enzymes which are playing an important role in physiological processes (Mudd, 1982; Guderian, 1985; Paoletti, 2007). Thus, stomatal closure can be considered as an avoidance mechanism of plants in response to high O_3 concentrations (Kolb and Matyssek, 2001). On the other hand, affected plants need photosynthate to construct and maintain their defense and repair systems (Kolb and Matyssek, 2001; Andersen, 2003; Wieser and Matyssek, 2007). Stomata must be open for CO_2 to enter. Therefore, a tradeoff between an increased carbon gain and a reduced O_3 uptake becomes a critical mechanism for plants to either avoid or tolerate adverse O_3-induced effects.

Because many biotic and abiotic factors affect plant gas exchange, plant response to O_3 is likely confounded with these factors as well (Matyssek and Sandermann, 2003; Paoletti, 2007), which may include tree age, genetic make-up, pests, competition, soil moisture, humidity, light intensity, temperature, and other co-occurring greenhouse gases (Reich, 1984; Karnosky et al., 2007). For example, plants tend to close their stomata to conserve water in response to water stress (Kramer, 1983) and thereby coincidentally avoid O_3 uptake (Reich, 1987; Matyssek and Sandermann, 2003). Previous studies have demonstrated that more O_3 uptake and more severe leaf injury of forest trees were correlated with a higher availability of soil water (Showman, 1991; Hildebrand et al., 1996;

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Chappelka and Samuelson, 1998; Grulke et al., 2003; Schaub et al., 2003; Ribas et al., 2005; McLaughlin et al., 2007). Although some studies have failed to demonstrate an effect of soil water availability on foliar injury (Heagle et al., 1988; Temple and Benoit, 1988), soil water availability by itself or additional other interacting factors are likely to complicate such comparisons (Grulke et al., 2003; Schaub et al., 2005; McLaughlin et al., 2007).

Leaf nitrogen concentration (N_L) is another factor that significantly influences photosynthetic gas exchange (Field and Mooney, 1986). Its effects on plant responses to O_3 have similar tradeoffs as water does (Greitner and Winner, 1989; Pell et al., 1990; Landolt et al., 1997). Because at least 75% of total N_L is found within compounds that relate to the biochemical and photobiological processes of photosynthesis, N perhaps is the most important factor in replacement of mesophyll tissue damaged by O_3 (Grulke, 2003). Trees with higher N_L seem to have higher photosynthetic rates, accompanied with higher stomatal conductance, and subsequently higher O_3 uptake, suggesting that plants with a higher N_L should be more sensitive to O_3 (Pell et al., 1990). However, a high supply of N tends to reduce O_3 injury and premature leaf loss in birch (Paakkonen and Holopainen, 1995). Therefore, N effects on the sensitivity of trees to O_3 are inconsistent (Greitner and Winner, 1989), which might be confounded by processes of leaf senescence and nutrient retranslocation (Matyssek and Sandermann, 2003).

The objective of this study was to examine the inter-relationships among leaf age, N_L, leaf gas exchange, and O_3-induced injury on black cherry (*Prunus serotina* Ehrh.) seedlings grown within open-top chambers. We tested the hypothesis that net photosynthesis, stomatal conductance, and N_L are strongly interrelated and collectively influence the incidence and severity of O_3-induced foliar injury. Using these data, we tried to determine whether we can find a mechanistic way to assess O_3 risk for forest seedlings. We used black cherry because: (1) it is one of the tree species native to the northeastern USA that is most sensitive to O_3 (Davis and Skelly, 1992; Simini et al., 1992); (2) it has a relatively high gas exchange rate (Fredericksen et al., 1995, 1996a, 1996b); (3) it has been extensively studied both *in situ* and in bio-indicator gardens (Simini et al., 1992; Kouterick et al., 2000; Ferdinand et al., 1999; Lee et al., 1999, 2002; Schaub et al., 2003, 2005); and (4) it is a very valuable commercial timber species (Marquis, 1990).

2. Materials and methods

2.1. Study site and experimental design

The study site was located at the Pennsylvania's Bureau of Forestry's Penn Nursery, (40° 40′ N latitude, 77° 37′ W longitude, 490 m a.s.l.) in Centre County, Pennsylvania, USA. Soils are Laidig series in the taxonomic subgroup Typic Fragidults, 0.6–1.8 m deep over colluvial material from sandstone and siltstone (USDA, 1981). The climate is a composite of relatively dry Midwestern continental climate. Maximum and minimum air temperatures and precipitation during study in 1998 are shown in Fig. 1. The experimental design consisted of three blocks with each block split into two water regimes with two open-top chambers (for controlling ozone exposure) and one open-plot (AA: ambient air) randomly arranged within each water regime (Schaub et al., 2003). One of each pair of chambers was supplied with carbon-filtered air containing approximately 50% of ambient O_3 concentrations (CF: ca. 0.5 × AA) and the other one was supplied with non-filtered air (NF: ca. 0.96 × AA). The entire experiment consisted of a total of 18 plots (6 AA + 6 CF + 6 NF), all of which received natural rainfall and nine of which were supplementary irrigated every 2–5 days to any drought effects. Each plot included four black cherry seedlings from each of four different, open-pollinated families (2 sensitive and 2 tolerant to O_3; see Lee et al., 1999), 10 white ash (*Fraxinus americana* L.), and 10 red maple (*Acer rubrum* L.) seedlings that were planted within the well-tilled nursery soil during late September 1997. The open-top chambers were operated throughout the growing seasons of 1998 and 1999. Seasonal and annual assessments for seedling phenology, growth, physiology, and typical ozone-induced symptoms were conducted during both growing seasons (Schaub et al., 2003).

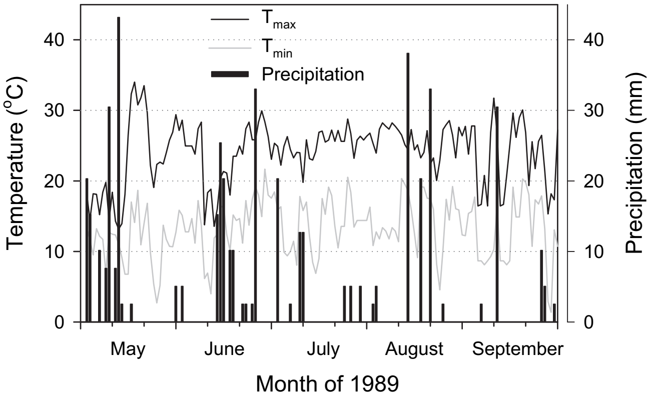

Fig. 1. Daily maximum and minimum air temperature and precipitation at the Penn Nursery site from May to September, 1998.

2.2. Physiological measurements

In this study and in respect to the hypothesis as outlined above, we used a sampling procedure that differed from the one as applied by Schaub et al. (2003). We used black cherry seedlings to study the effect of N_L and leaf age on the leaf gas exchange to O_3. We randomly chose one seedling per family within each plot during each of seven sampling dates for the physiological study from July 30 to August 7, 1998. During each sampling day, we measured a complete block of six plots and rotated within three blocks. Four leaves of one seedling were randomly selected within each of four age-classes from the youngest (1st age-class) to the oldest leaf (4th age-class). When we started to monitor O_3 on 16 May 1998, we identified and tagged 48 unfold leaves as our oldest leaves. The other leaf age-classes were determined based on leaf position and shoot growth rate between the oldest leaf position and the current shoot tip. On each sampling date, the leaves of the oldest 4th age-class were 11 weeks old, 3rd age-class ca. 7 weeks old, 2nd age-class ca. 4 weeks old, and the youngest, the 1st age-class, ca. 2 weeks old (and not yet fully expanded).

Net photosynthesis (A) and stomatal conductance to water vapor (g_{wv}) were measured under constant light conditions (PAR = 1200 μmol m^{-2} s^{-1}) with a Li-Cor 6400 portable photosynthesis system with a red/blue LED light source and CO_2 injector (Li-Cor Inc. Lincoln, Nebraska, USA). Measurements took place from 0900 to 1200 h and from 1300 to 1600 h on sampling days. The temperature within the cuvette was maintained near ambient air temperature, which varied between 24 and 35 °C depending on time of day and the day when measurements were taken. A constant CO_2 concentration at 355 μmol mol^{-1} was supplied with a fixed air flow of 500 μmol s^{-1}. Pertinent environmental variables were closely monitored including air temperature, relative humidity, and vapor pressure deficit.

Light-response curves were measured by setting photosynthetically active radiation (PAR) from 1400 to 1200, 1000, 800, 600, 400, 200, 100, 50, and 0 μmol m^{-2} s^{-1}. We let the leaves acclimate to the preset light conditions within the cuvette for 3 min before each measurement. We measured light-response curves on four healthy leaves from the 2nd age-class and on four leaves with O_3-induced symptoms from 3rd to 4th age classes within NF chambers either within irrigated or in non-irrigated regimes. We fit these data with the non-rectangular hyperbolic model developed by Hanson et al. (1987). We then estimated light-saturated maximum assimilation rates (A_{max}), light compensation points (Γ), dark respiration (R_d), and quantum yield (Φ).

2.3. Leaf nitrogen concentration

After leaf gas exchange was measured, we collected four leaves from each age-class per family within each of the 18 plots. These leaves were ground and N_L (%) was determined by combustion with Fisons Elemental Analyzer (Fisons, Italy) within the Agricultural Analytical Services Laboratory, Pennsylvania State University.

2.4. O_3-Induced injury evaluation

We visually rated the percentage adaxial stipple on individual leaves that we used for gas exchange measurements. The Horsfall-Barratt rating system (Horsfall and Barratt, 1945) developed for the Forest Health expert system (Nash et al., 1992) was used to determine an average percent leaf area being affected (%) by visible O_3-induced injury. Ratings were recorded as 0, 3, 6, 12, 25, 50, 75, 88, 94, 97, and 100% leaf area affected. Other than in Schaub et al. (2003), these leaves were individually selected and rated and represent their respective age-class as selected for this study only.