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Effects of a three-year exposure to ambient ozone on biomass allocation in poplar using ethylenediurea

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ABSTRACT

We examined the effect of ambient ozone on visible foliar injury, growth and biomass in field-grown poplar cuttings of an Oxford clone sensitive to ozone (*Populus maximoviczii* Henry × *berolinensis* Dippel) irrigated with ethylenediurea (EDU) or water for three years. EDU is used as an ozone protectant for plants. Protective effects of EDU on ozone visible injury were found. As a result, poplar trees grown under EDU treatment increased leaves, lateral branches and root density in the third year, although no significant enhancement of stem height and diameter was found. Ambient ozone (AOT40, 24.6 ppm h; diurnal hourly average, 40.3 ppb) may finally reduce carbon gain by reducing the number of branches, and thus sites for leaf formation, in ozone-sensitive poplar trees under not-limiting conditions.

1. Introduction

Tropospheric ozone (O_3) is a widespread phytotoxic air pollutant, and a major concern for forests (Bytnerowicz et al., 2007; Serengil et al., 2011). Background concentrations of O_3 in the troposphere have continuously increased since the pre-industrial age, although control measures on precursors emission have reduced O_3 peaks (Vingarzan, 2004; Derwent et al., 2007). A continuous increase in background O_3 concentrations in the northern hemisphere is predicted in the near future (Royal Society, 2008). Ambient ozone levels have thus the potential to cause a negative impact on forest trees. Ozone may cause growth reduction, impairment of physiological traits, such as leaf gas exchange, and foliar injury, i.e., interveinal dark stippling on the leaf surface (Paoletti, 2007).

Ozone visible injury has been investigated in more than 75 European and 66 North American species of native and exotic trees, shrubs and herbs, and partly validated under controlled conditions (e.g., Innes et al., 2001; Paoletti et al., 2009a,c). After onset of O_3 visible injury, significant reductions in steady-state leaf gas exchange were recorded for tree species in chamber experiments (Zhang et al., 2000; Paoletti et al., 2004; Novak et al., 2005). Maintenance of O_3-injured leaves may raise respiratory costs for repair of the damaged parts (Matyssek and Sandermann, 2003). Predictions have been made that O_3 exposure causes forest growth losses (Broadmeadow, 1998; Chappelka and Samuelson, 1998; Ollinger et al., 1997). Verifying the O_3-induced loss in forest trees is difficult. It has been ascertained in tree seedlings, cuttings or young trees using ozone fumigation, or ambient air compared to filtered air, in open top chambers or free-air fumigation (Matyssek and Sandermann, 2003; Karnosky et al., 2005).

Ethylenediurea (EDU, N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea) has been shown to protect leaves from O_3 injury (reviewed by Manning et al., 2011) and has been used in the field (e.g., Paoletti et al., 2007a,b). EDU can in particular be applied systemically by watering and may meet a cocktail of secondary toxicants induced by ozone in the apoplast (Manning et al., 2011). Evidence has been given that EDU acts in the apoplast as a potential chemical scavenger (Gatta et al., 1997), thus protecting leaves from ozone injury while photosynthesis is not changed (Feng et al.,

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2010). The application of EDU as a 'control' to ambient ozone therefore may determine ambient ozone effects in field grown plants (Manning et al., 2011). However, only two >2-yr EDU studies have been conducted on trees until now (Long and Davis, 1991; Manning et al., 2003). Smaller basal diameter (−11%) and lower above-ground biomass (−28%) was found in non-EDU treatment relative to EDU treatment of 450 ppm for loblolly pine (*Pinus taeda* L.) in east Texas, using biweekly sprays of EDU over three years (Manning et al., 2003). Also, lower above ground biomass (−32%) was observed in non-EDU treatment than EDU treatment of 1000 ppm for *Prunus serotina* Ehrh in Pennsylvania, using foliar spray treatments over four years (Long and Davis, 1991).

In the present study, we examined the visible foliar injury, biomass accumulation and allocation, including roots, in field-grown poplar trees treated with EDU or not, and discussed the effects of ambient O_3 (versus a 'control' protected by EDU) for three years in central Italy on the poplar clone known for its sensitivity to O_3.

2. Materials and methods

2.1. Plant material and site description

The study was carried out in a former agricultural site located in central Italy (Antella: 43°44′ N, 11°16′ E, 50 m a.s.l., 15.8 °C as mean annual temperature). Forty root cuttings of an O_3-sensitive Oxford clone (*Populus maximoviczii* Henry × *berolinensis* Dippel) were planted in two lines (20 cuttings in each line) in autumn 2007 with a spacing of 1 m between trees along a line and of 3 m between trees of the two lines. A detailed description of the experiment is in Hoshika et al. (2012). Every week over the growing seasons (April to October), each tree was drip irrigated with 1 (in 2008) to 2 L of water (in 2009 and 2010) to avoid water stress. No EDU treatment (WAT, control line) or 450 ppm EDU solution (EDU, treated line) was applied, according to the successful application of 450 ppm EDU to tree species (Paoletti et al., 2009a) and as soil drench to adult ash trees (Paoletti et al., 2011). Repeated application of EDU is necessary because of degradation over time (Carnahan et al., 1978) and no mobilization from old tissues to new sprouts (Weidensaul, 1980). Soil moisture was measured in the root layer (30 cm depth) by EC5 sensors equipped with an EM5b data logger (Decagon Devices, Pullman WA, USA). On average, soil moisture was 24.5 ± 0.1% since April to October (growing season). The values were near to field capacity (25.5%) for this type of soil, i.e. sandy clay loam. Air temperature, relative humidity and precipitation were recorded by a 110-WS-16 modular weather station (NovaLynx corp., Auburn CA, USA). Ozone concentrations were recorded at canopy height by an annually-calibrated O_3 monitor (Mod. 202, 2B Technologies, Boulder CO, USA). Ozone exposure indices were calculated during the growing season (April to October) as: AOT40, i.e. the accumulated exposure above a threshold concentration of 40 ppb during daylight hours; M24, i.e. daily average over the 24 h; M12, i.e. diurnal average over the time window 8 am to 8 pm CET.

2.2. Measurements of plant growth and biomass

Measurements of tree height (by a 0.5-cm graduated mast) and diameter (at the collar when plants were <2 m height, and at breast height when plants were >2 m height) were carried out in September from 2008 to 2010. Relative growth rate (RGR) for height (cm cm^{-1} year^{-1}) was assessed as $[(\ln H_2 - \ln H_1)/(t_2 - t_1)]$, where H denotes height (cm) and t denotes the year. The subscript numbers indicate the sampling time (i.e., "1" and "2" indicate initial and final samplings, respectively). The number of leaves was counted in the same trees in September and October 2008 to 2010. The plants did not show significant differences in stem diameter, height, and number of lateral branches at the beginning of the experiment (data not shown).

Five trees in each treatment were selected at random, harvested and separated into each organ (i.e., stem, branch and leaf) at the end of the experiment (early October 2010). The number of lateral branches and branch length were measured. Mean single leaf area was measured for 30 fully expanded leaves per tree by scanning and calculating the area using a AM300 area meter (ADC, BioScientific Ltd, Herts UK). Fine roots (diameter: <2 mm) were sampled in standard cores of soil (500 ml). Four cores per tree were collected in the upper 20-cm of soil, 25 cm far from the trunk along the four directions (north, south, east and west), and joined in a single sample. Poplar fine roots were gently washed and separated from the roots of herbaceous species by means of visual inspection. When needed, thin sections were cut and classified according to Agerer (1987–2002). Course roots (diameter: >2 mm) were extracted by digging additional soil cores (60 cm depth × 280 cm between the tree lines × 100 cm along a line) localized around the base of a trunk, that was cut at the collar. Dry mass of each plant organ was determined by oven-drying at 65 °C until a constant weight was reached.

2.3. Measurements of total nitrogen content

Randomised sub-samples of oven-dried leaves, branches, stems, coarse roots and soil were pooled in four samples. Total nitrogen content was determined using two methods: i) dry combustion and ii) modified Kjeldahl method. In the former method, the sample was heated to a temperature of at least 900 °C in the presence of oxygen. The content of total nitrogen was measured using thermal conductivity with a LECO TruSpec C/N analyzer (Cools and De Vos, 2010; ISO 13878, LECO Corporation, 2006). In the latter method, titanium dioxide was used as catalyst instead of selenium (classic Kjeldahl method), distillation apparatus being Gerthardt (Cools and De Vos, 2010; ISO 11261).

2.4. Assessment of ozone visible injury

Plants were periodically surveyed over the growing seasons. Ozone visible injury occurred as dark stippling on the upper leaf surface (Fig. 1) since early September every year. The injury was identified as O_3-like because it was missing in shaded leaves and apparently more severe in older than in younger leaves (Innes et al., 2001). The symptoms were similar to those caused by ambient O_3 in *Populus nigra* (Novak et al., 2005). In September 2008 to 2010, the same two observers counted the number of leaves within each injury class (5%-step) for all the trees per treatment (WAT and EDU). Given the large size of the plants, in 2010 the survey was feasible only for a limited number of plants ($N = 3$) and was thus repeated at early October. Photoguides where visible injury was quantified by image analysis processing were used (Innes et al., 2001; Paoletti et al., 2009b). Pest, pathogen and mechanical injury occurred in both EDU and WAT trees and were assessed to be <5% of total leaves. The number of symptomatic leaves was expressed as percentage of ozone-injured leaves per tree (LA), and the percent surface injury was expressed as average percentage of ozone-injured leaf surface per symptomatic leaf (AA). A Plant Injury Index (PII) was calculated combining these two parameters in each tree: $PII = (LA \times AA)/100$, according to Paoletti et al. (2009b).

2.5. Statistics

Data were tested for normal distribution (Kolmogorov–Smirnov D-test) and homogeneity of variance (Levene's test). The effects of ambient O_3 on plant biomass, nitrogen content and O_3 visible injury were tested via Student *t*-test or Mann–Whitney's *U*-test. Result was considered significant at $p \le 0.05$. All statistical analyses were performed with SPSS software (20.0, SPSS, Chicago, USA).

Fig. 1. Example of leaves with no visible ozone injury (left) and 15% injury (right).