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Chronology of Changes within the Photosynthetic Apparatus of *Populus nigra* under Ozone Stress

By

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Summary

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The sequence of changes within the photosynthetic apparatus of *Populus nigra* leaves, fumigated with ozone in open-top chambers, was investigated. Besides two chambers with charcoal-filtered air (CF), two with non filtered air (NF) and two with non filtered air +50 nl l^{-1} ozone (NF+) were used. The first effect to be found on photosynthesis was a significant reduction in carboxylation efficiency (CE) in 10 day old NF- and NF+-leaves compared to CF-leaves. This effect was enhanced with the ageing of the leaves. On the other hand photochemical capacity measured by dark-adapted chlorophyll fluorescence (F_v/F_m) was not reduced in NF-leaves compared to CF-leaves during the whole growing season. In NF+-leaves F_v/F_m showed a decrease in comparison to CF-leaves from 42 days onwards, when CE was already reduced by about 60% in the same leaves. In 34 and 75 day old leaves there was an increase in the ratio of the xanthophylls violaxanthin + antheraxanthin + zeaxanthin to chlorophyll a+b in leaves that were exposed to elevated ozone concentrations indicating enhanced light stress in these leaves.

Introduction

Ozone is regarded as one of the most phytotoxic air pollutants (LEFOHN 1992). Ozone induced reduction in the rate of photosynthesis has been claimed to a reduction in stomatal conductance (HILL & LITTLEFIELD 1969, GREITNER & WINNER 1988, MARTIN & al. 1988) and to impairment of biochemical events in the mesophyll (REICH & LASSOIE 1984, WALLIN & SKÄRBY 1992, NIE & al. 1993,

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COULSON & HEATH 1974, SCHREIBER & al. 1978). In a comprehensive study GUZY & HEATH 1993 found variable reactions in photosynthetic rate, stomatal conductance and biochemical parameters in several cultivars of common bean. This shows that there is obviously not a unique mode of action of ozone on plant cells.

Presently it is generally assumed that ozone forms oxygen radicals which react initially with biomolecules like ascorbic acid (LEE & al. 1984, CASTILLO & GREPPIN 1988) in the cell wall and phospholipids in the plasmalemma (FANGMEIER & al. 1994). Since ozone and the oxygen radicals may react with almost every cell components, we were interested in the question, which part of the photosynthetic apparatus of a leaf was damaged first. Thus we measured the carboxylation efficiency (CE) with the aid of CO_2-response curves and the photochemical capacity of PSII with the aid of chlorophyll fluorescence measurements after dark adaption (F_v/F_m) and the pigment content of leaves by HPLC.

Materials and Methods

Cuttings of *Populus nigra* (Clone T 107) were planted into 6 l-pots in a fertilised potting compost (Frux ED 63, N:P:K = 1:1:1) in spring 1992. In April 1993, when plants were about 1.5 m in height, plants were transfered into 12 l-pots with Frux ED 63. Six plants were put in every open-top chamber at the beginning of May. Three treatment groups with two chambers per group were investigated: Charcoal filtered air (CF), non filtered air (NF) and non filtered air with addition of 50 nl l^{-1} ozone (NF+). Ozone was produced by an ozone generator (Fischer, Mod. 502, Meckenheim/Bonn, Germany) using pure oxygen and was supplied from Monday to Friday (9 a.m. to 5 p.m.).

The OTCs were constructed, as described by HEAGLE & al. 1973 with a diameter of 3 m and a height of 2.4 m. The air volume of the chambers was exchanged 2.5 times a minute, to equilibrate air temperature and humidity with the conditions outside. Nevertheless there was an increase in air temperature inside the chambers compared with outside conditions between 1.1 and 2 °C. The mean ozone concentration (day and night) during the exposure period (3rd May to 10th September 1993) in the CF-chambers was 10 ± 3 nl l^{-1}. In the NF-chambers the mean concentration was 38 ± 10 nl l^{-1} and in the NF+-chambers it was 55 ± 16 nl l^{-1}. Peak concentrations were 23 nl l^{-1} in the CF-chambers, 93 nl l^{-1} in the NF-chambers and 143 nl l^{-1} in the NF+-chambers. In NF-chambers the leaves under investigation had experienced an ozone dose (AOT40) of 5 ppm-h after 34 days and of 9 ppm-h after 75 days. In NF+-chambers the corresponding values were 14 ppm-h and 25 ppm-h. In the CF-chambers the ozone concentration never reached 40 ppb.

Measurements were made on the same leaves during the whole experiment (leaves were marked with a wool thread). All gas exchange measurements were performed between 10 a.m. and 3 p.m. using a portable gas exchange analyser (LICOR 6200, Lincoln, Nebraska, USA). CO_2-response curves were made under saturating light from ambient CO_2-concentration down to the CO_2-compensation point as described by MCDERMIT & al. 1989. Measurement of one CO_2-response curve lasted between 30 and 45 minutes. Therefore only six curves could be measured between 10 a.m. and 3 p.m.

Measurements of chlorophyll fluorescence were made with a portable fluorimeter ("Plant Efficiency Analyser", PEA, Hansatech Instruments Ltd, King's Lynn, UK). Leaves were dark adapted for 30 minutes prior to measurements. The value F_v/F_m was designated as photochemical capacity (BOLHÀR-NORDENKAMPF & ÖQUIST 1993), characterising the photochemical acitvity of PS II.

For pigment analysis whole leaves were harvested and transferred to a refrigerator at - 80 °C. The duration between harvesting and storing was exactly 20 minutes. Leaves were then freeze-dried at -1 °C and 100 mbar (Hetosicc CD4, Hetolab Equipment) for three days, homogenised (Micro-Dismembrator II, Fa. Braun) and again stored at -80 °C. Extraction of pigments and HPLC-analysis was performed as described by PFEIFHOFER 1989.

Results and Discussion

During the leaf development the first effect to be found was a reduction of the initial slope of A/c_i-curves in 10 days old NF- and NF+-leaves compared to CF-leaves (Fig. 1). This initial slope is correlated with the carboxylation efficiency (CE) of the dark reaction of photosynhtesis (VON CAEMMERER & FARQUHAR 1981). A reduction in CE under ozone exposure was already shown for poplar (MATYSSEK & al. 1993) The reduction in CE with increasing ozone concentration became more enhanced with increasing leaf age. The reduced CE was not due to a limitation of CO_2-influx into the intercellular space, since stomatal conductance showed no significant differences between the three treatment groups (data not shown). In NF+-plants there was a premature shedding of leaves. After 75 days only one leave of the initially twelve marked leaves of this group was left.

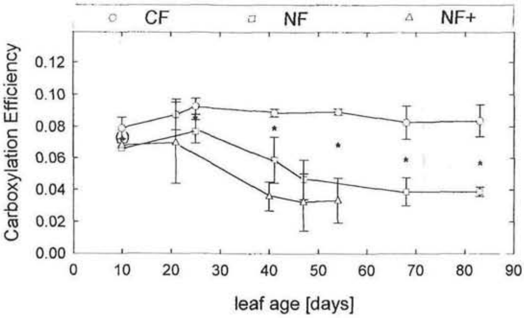

Fig. 1. Carboxylation efficiency of leaves of *Populus nigra* exposed to different concentrations of ozone - calculated from the initial slope of A/C_i-curves. Asterisks between symbols indicate significant differences. (Duncan Test; (*)...$p < 0.1$, *...$p < 0.05$; $n = 3$). CF...charcoal filtered, NF...non filtered, NF+...non filtered + 50 nl l^{-1} ozone. NF+-leaves were shed prematurely, on day 75 only one of the marked leaves was left.

Though in NF-leaves CE was reduced compared to CF-leaves there was no reduction in photochemical capacity (F_v/F_m) during the whole experiment (Fig. 2). In NF+-leaves F_v/F_m showed a decline on day 42 compared to CF-leaves. At that time CE was already decreased by about 60% in the same leaves.

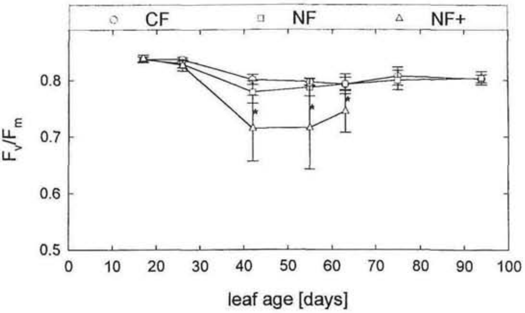

Fig. 2. Photochemical capacity of leaves of *Populus nigra* exposed to different concentrations of ozone measured by chlorophyll fluorescence after dark adaption (F_v/F_m). Asterisks between symbols indicate significant differences. (Duncan Test, *....$p < 0.05$, $n = 12$). CF...charcoal filtered, NF...non filtered, NF+...non filtered + 50 nl l^{-1} ozone. NF+-leaves were shed prematurely, on day 75 only one of the marked leaves was left.

These data confirm findings of LEHNHERR & al. 1988, FARAGE & al. 1991 and GUPTA & al. 1991 that the dark reaction of photosynthesis is impaired by ozone before effects on the light reaction can be detected. Since the dark reaction and the light reaction are linked by the two molecules ATP and NADPH, which are produced in the latter and consumed in the former, an effect on the light reaction would be expected, if the dark reaction is impaired. Such an effect was found in the reduction of the maximal fluorescence (F_m) and the content of chlorophyll a+b (BALLACH & al. 1991) in leaves of NF- and NF+-leaves with increasing age compared to CF-leaves (Fig. 3 A, B). In NF-leaves F_m and the content of chlorophyll a+b decreased by about the same percentage compared to CF-leaves. In NF+-leaves F_m decreased by a smaller percentage than the content of chlorophyll a+b, indicating a change in the composition of PSII (KRAUSE & WEIS 1991) under elevated ozone concentrations.

In 10 day old leaves the ratio V+A+Z/Chl a+b was lower in NF+-leaves compared to CF and NF-leaves. In 34 day and 75 day old leaves this ratio was increased in NF- and NF+-leaves compared to CF-leaves. An increased ratio of V+A+Z/Chl a+b is characteristic for leaves that are exposed to light levels that override the capacity of the electron transport chain (DEMMIG-ADAMS & ADAMS 1992, ROBINSON & al. 1993). Thus the increase of this ratio in 34 and 75 day old leaves was presumably caused by excess light energy due to a reduced demand of ATP and NADPH in the calvin cycle. In 10 day old NF+-leaves the ratio V+A+Z/Chl a+b was reduced. At that time there was obviously no excess light, maybe because of a higher demand of energy for repair processes caused by ozone (compare GUPTA & al. 1991).

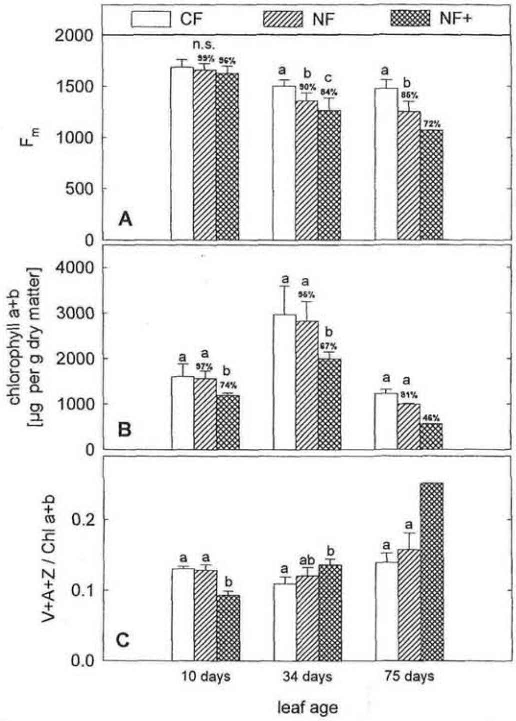

Fig. 3 A-C. (A) Maximal fluorescence measured after dark adaption (n = 12, except NF+/75 days: n = 1) (B) total chlorophyll content of leaves on a dry weight basis (n = 4, except NF+/75 days: n = 1) (C) the ratio of the xanthophylls violaxanthin + antheraxanthin + zeaxanthin to the content of chlorophyll a+b (n = 4, except NF+/75 days: n = 1). All data are given in mean ± s.d. Letters above the bars indicate significant differences between treatments (Duncan Test, $p < 0.05$). In (A) and (B) values are also indicated as percentage of CF-leaves. CF...charcoal filtered, NF...non filtered, NF+...non filtered + 50 nl l^{-1} ozone.

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