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Ozone-induced changes in carotenoids and chlorophylls in three *Populus* clones

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Introduction

Ozone is a phytotoxic air pollutant causing oxidative stress. We studied the effect of ozone on carotenoids, chlorophylls and polyisoprenoid alcohols in three euramerican poplar clones (*Populus deltoides* x *Populus nigra*: Carpaccio, Cima and Robusta).

Materials and Methods

Poplars originating from cuttings were grown in a growth chamber for 5 weeks and acclimated for a week in fumigation chambers at 23/20 °C (14/10 h day/night). Four fumigation chambers were used for each treatment: control and ozone fumigation (120 ppb each day for 13 h). The 10th and 11th leaf calculated from the top were collected 2, 4, 11, 15 and 17 days after the start of the ozone treatment. Chemical analyses were made of freeze-dried samples extracted by MeOH and hexane (containing butylated hydroxytoluene and trans-8'-apo-beta-caroten-8'-al) by HPLC-API-MS. The compounds were tentatively identified by MS/MS and UV spectra.



Figure 1. *Populus* genotypes (Carpaccio, Cima and Robusta) at day 17 of the ozone treatment.



Figure 2. Visible injuries caused by ozone in *Populus* genotypes (Carpaccio, Cima and Robusta) at day 17 of the ozone treatment.

Table 1. Tentatively identified compounds and their identification details.

rt	Compound	Quan [M+H] ⁺	Absorption peaks	%III/II	%AB/II	
1.04	Unknown 1	609	609			
1.21	Unknown 2	611				
1.22	All-trans-neoxanthin	583	414, 438, 466	86		
1.28	All-trans-violaxanthin	601	418, 442, 470	92		
1.37	Unknown 3	611	611			
1.38	Luteoxanthin-type	601	402, 422, 450	98		
1.53	Cis-violaxanthin	601	330, 416, 436, 464	19	35	
1.54	Unknown 4	611	611			
1.72	All-trans-lutein	551	569	422, 446, 474	56	
1.76	Unknown 5	613	613			
2.04	Cis-lutein-type	551	569	332, 420, 442, 468	31	46
2.13	Unknown 6	613	613			
2.3	ISTD	417	417	458	0	
2.67	Unknown 7	591				
2.97	Unknown 8	397				
3.31	Unknown 9	591	591			
3.72	Chlorophyll b	907	907	464, 644		
5.41	Chlorophyll a	893	893	432, 662		
5.86	Unknown 10	397				
7.91	All-trans-beta-carotene	537	537	425, 454, 480	14	
8.08	9-cis-beta-carotene			340, 422, 450, 476	9	5
8.3	13/15-cis-beta-carotene	537	537	340, 420, 446, 464	0	28
10.65	Phaeophytin a	871	871	408		

Quan = ion used for quantification

Ozone reduced *Populus* growth

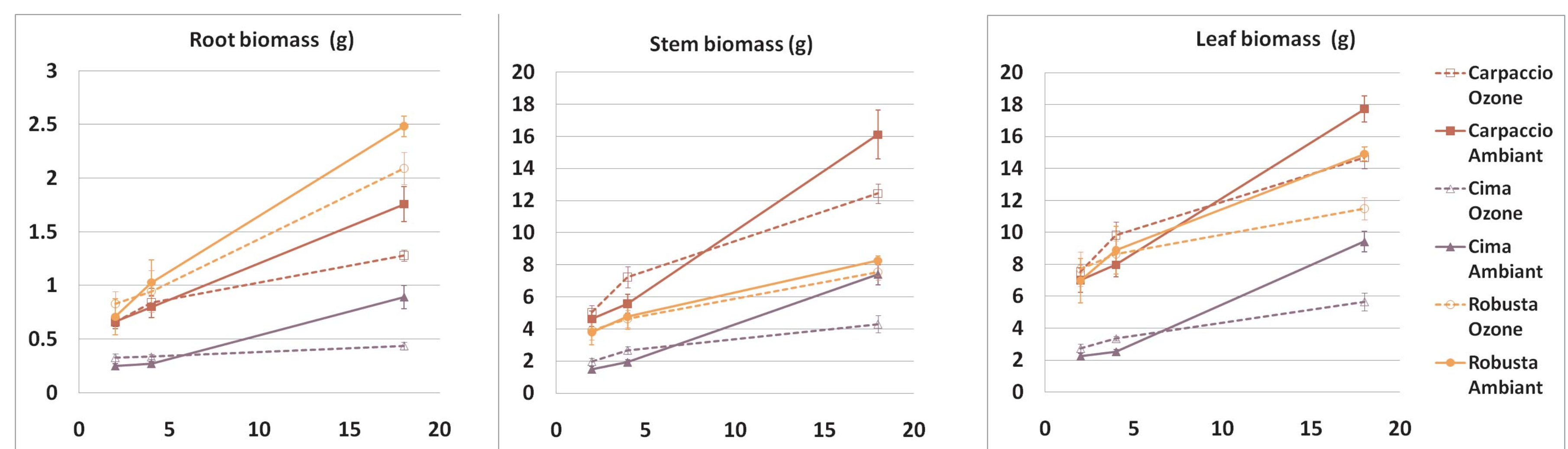
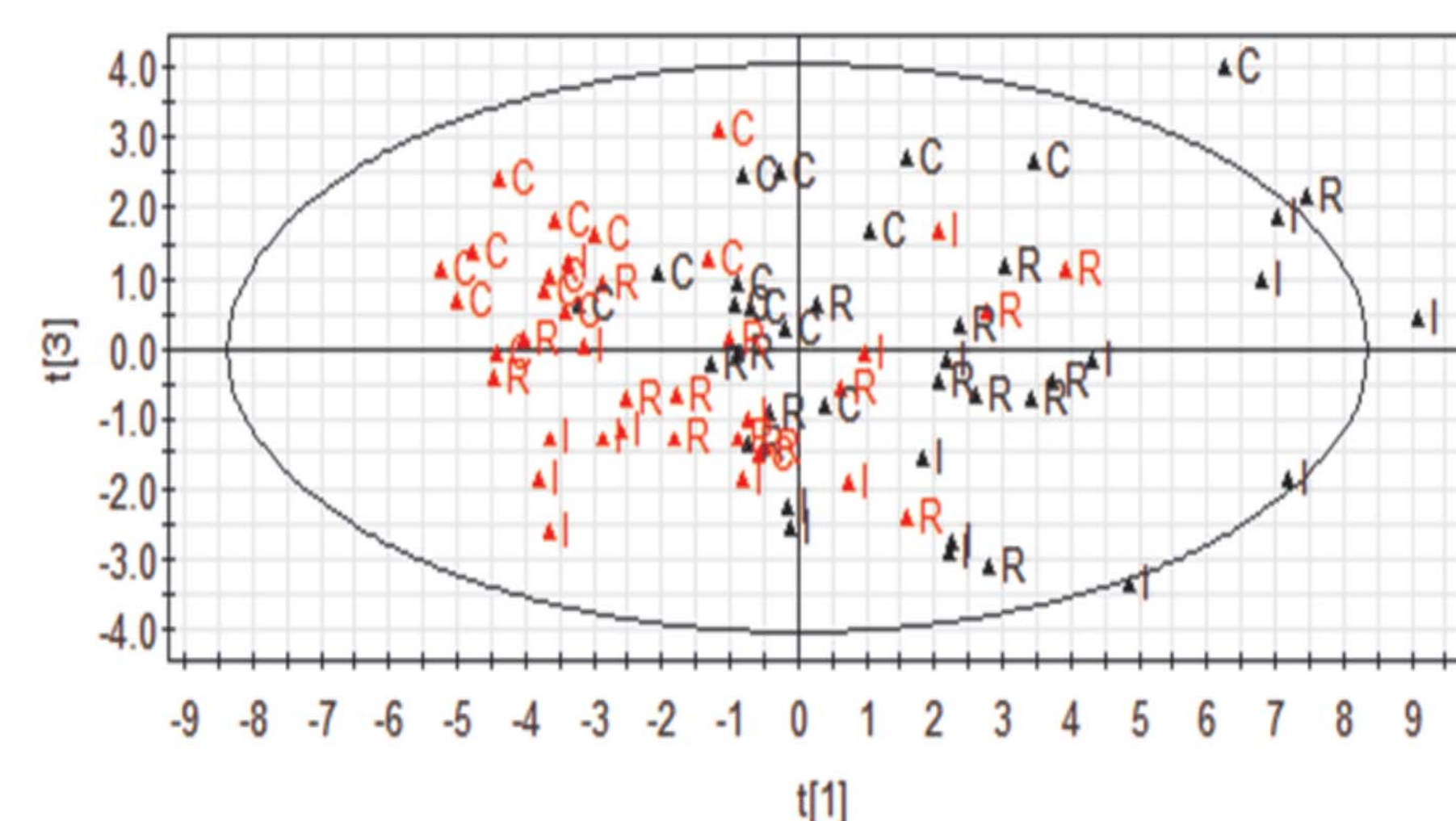


Figure 3. Biomass accumulation (mean, s.e.) in different plant parts during the duration of the experiment (days).

Carpaccio had the highest above-ground and total biomass both in control and ozone-treatment. Ozone reduced the growth of Cima the most, while Robusta showed the greatest number of visible injuries.

Ozone reduced accumulation of carotenoids, chlorophyll and polyisoprenoid alcohols



Accumulation reduced by ozone:
 Chlorophyll a
 Chlorophyll b
 All-trans-lutein
 Cis-lutein-type
 All-trans-beta-carotene
 9-cis-beta-carotene
 13/15-cis-beta-carotene
 All-trans-neoxanthin
 6 currently unknown polyisoprenoid alcohols

Figure 4. Principal component analysis based on the carotenoids, chlorophylls and polyisoprenoid alcohols analysed. Red = ozone-treated, black = control. C = Carpaccio, I = Cima, R = Robusta. List of compounds that were reduced by ozone.

The ozone and control samples were clearly separate in principal component analysis (PCA) based on the analysed chemical profiles. Ozone treatment explained 50 % of the variance in the data.

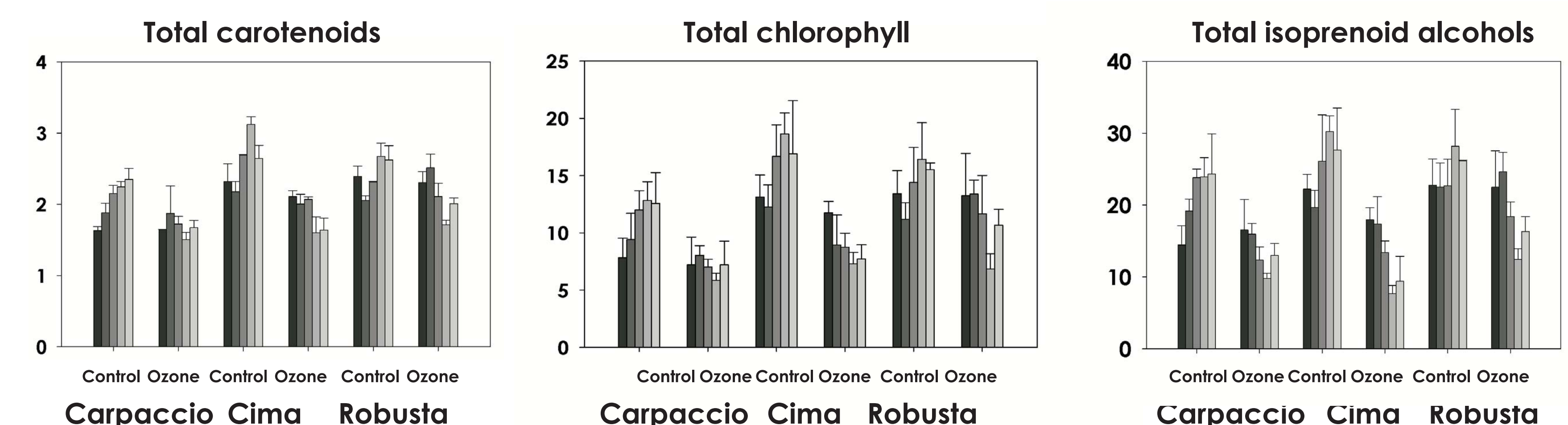


Figure 4. Total carotenoids, chlorophyll (including pheophytin a) and polyisoprenoid alcohols (Mean, s.e.)/arbitrary units proportional to the ISTD trans-8'-apo-beta-caroten-8'-al on dry weight basis.

Carotenoids, chlorophylls and polyisoprenoid alcohols were reduced by ozone. The responses were similar in all genotypes, despite the differences in tolerance between the clones.

In conclusion:

Ozone tolerance in *Populus* appears to be mediated by other factors than by carotenoids, chlorophylls or isoprenoid alcohols.