IMPACT OF GREENHOUSE GASES ON EPICUTICULAR WAXES OF *Populus tremuloides* M i c h x.: RESULTS FROM AN OPEN–AIR EXPOSURE AND A NATURAL O_3 GRADIENT

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Abstract

Maňkovská B., Percy K., Karnosky D.F.: Impact of greenhouse gases on epicuticular waxes of Populus tremuloides M i c h x.: results from an open–air exposure and a natural O_3 gradient. Ekológia (Bratislava), Vol.22, Supplement 1/2003, p.

Epicuticular waxes of three trembling aspen (*Populus tremuloides* M i c h x.) clones differing in O_3 tolerance were examined for three growing seasons (1998/2000) at three localities (Rhinelander, WI – clean and control site: Kalamazoo, MI – moderate pollution loading and Kenosha, WI – high pollution loading) in the Lake States region of the USA and at FACTS II (Aspen FACE) site in Rhinelander, WI. Differences in epicuticular wax structure were determined by scanning electron microscopy and quantified by a coefficient of occlusion. Statistically significant increases in stomatal occlusion occurred for the three O_3 bioindicator sites as we predicted with the higher O_3 sites having the most affected stomata for all three clones as well as for all treatments including elevated CO_2, elevated O_3, and elevated $CO_2 + O_3$. The results suggest that O_3 pollution of the Kenosha and Kalamazoo sites show significant negative impact on epicuticular waxes of aspen and these impacts are the most severe on the most O_3 sensitive clones. We recorded statistically significant differences between aspen clones, sampling period (spring, summer, fall) and localities Rhinelander, Kalamazoo and Kenosha. We found no statistically significant differences between treatments or aspen clones in stomatal frequency.

Key words: epicuticular wax, aspen, elevated O_3, elevated CO_2, interacting CO_2 and O_3

Introduction

Global atmospheric and pre–industrial CO_2 concentrations are expected to double by the end of the next century (Keeling et al., 1995). Troposphere ozone (O_3), a secondary pollut-

T a b l e 1. Classification of changes of the epistomatal wax of *P. tremuloides*

Class 1:	A maximum of 10% of the total stomatal area show the beginnings of fusion of single wax tubules
Class 2:	Several of the apically aggregated wax tubules fuse to small wax tufts at different parts of the epistomatal area. The latter cover 10% to 25% of the total stomata area
Class 3:	In addition to the wax tufts plate–like wax parts can be found that, in total, cover more than 25% and up to 50% of the total stomata area
Class 4:	More than 50% and up to 75% of the total stomata area show small parts of wax tufts as well as large platelet wax forms*Class 5:* More than 75% of the total stomata area is characterized by considerably changed wax microstructures, the stomata antechamber is almost or completely occluded with an amorphous wax plug

ant generated from nitrogen oxides (NO_x) and volatile organic compounds (VOC) from fossil fuel, such as thermal generation and transportation is also increasing globally. At the same time, forest tree species are exposed to the effect of CO_2, O_3 and other pollutants. While CO_2 generally stimulates tree growth and O_3 and other air pollutants generally decrease tree growth, there is little information available on the impacts of interaction of CO_2 and O_3 on epicuticular waxes and stomatal frequency. Trembling aspen is a good model species to examine the effects of these two pollutants, as it is highly responsive to both CO_2 and O_3. Furthermore, we have identified a wealth of genetic variation in the response of trembling aspen to air pollutants, and we have isolated O_3 – sensitive and tolerant clones (Karnosky et al., 1998, 1999).

Elevated CO_2 and O_3 affect trees through different mechanisms. With trembling aspen elevated CO_2 stimulates photosynthesis (Tjoelker et al., 1998; Noormets et al., 2001), delays foliar senescence in autumn and stimulates aboveground (Isebrands et al., 2001) and belowground (King et al., 2001) growth. Trees grown with elevated CO_2 generally have lower nitrogen concentrations in their foliage, lower Rubisco concentrations, altered defence compounds (Lindroth et al., 1993, 1997) and decreased concentration of antioxidants (Wustman et al., 2001).

In contrast to the largely beneficial effects of CO_2 on aspen, O_3 is generally detrimental to aspen growth and productivity. Ozone has been shown to induce foliar injury (Karnosky, 1976), decrease foliar chlorophyll content (Gagnon et al., 1992), accelerate leaf senescence (Karnosky et al., 1996), decrease photosynthesis (Coleman et al., 1995a), alter carbon allocation (Coleman et al., 1995b), alter epicuticular wax structure and composition (Maňkovská et al., 1999; Karnosky et al., 1999), and decrease growth (Wang et al., 1986; Karnosky et al., 1976, 1992, 1996, 1998a, 1998b; Coleman et al., 1996).

The overall objective of the work is to compare: a) the impacts of elevated levels of O_3, and CO_2 of on epicuticular wax formation; b) the seasonal development of stomatal frequency; c) the impact of a $1.5 \times O_3$ treatment in an open–air exposure system to that in a naturally high ambient O_3 area downwind of a major metropolitan area. In addition we examined the effects of elevated O_3 and CO_2 on stomatal frequency.

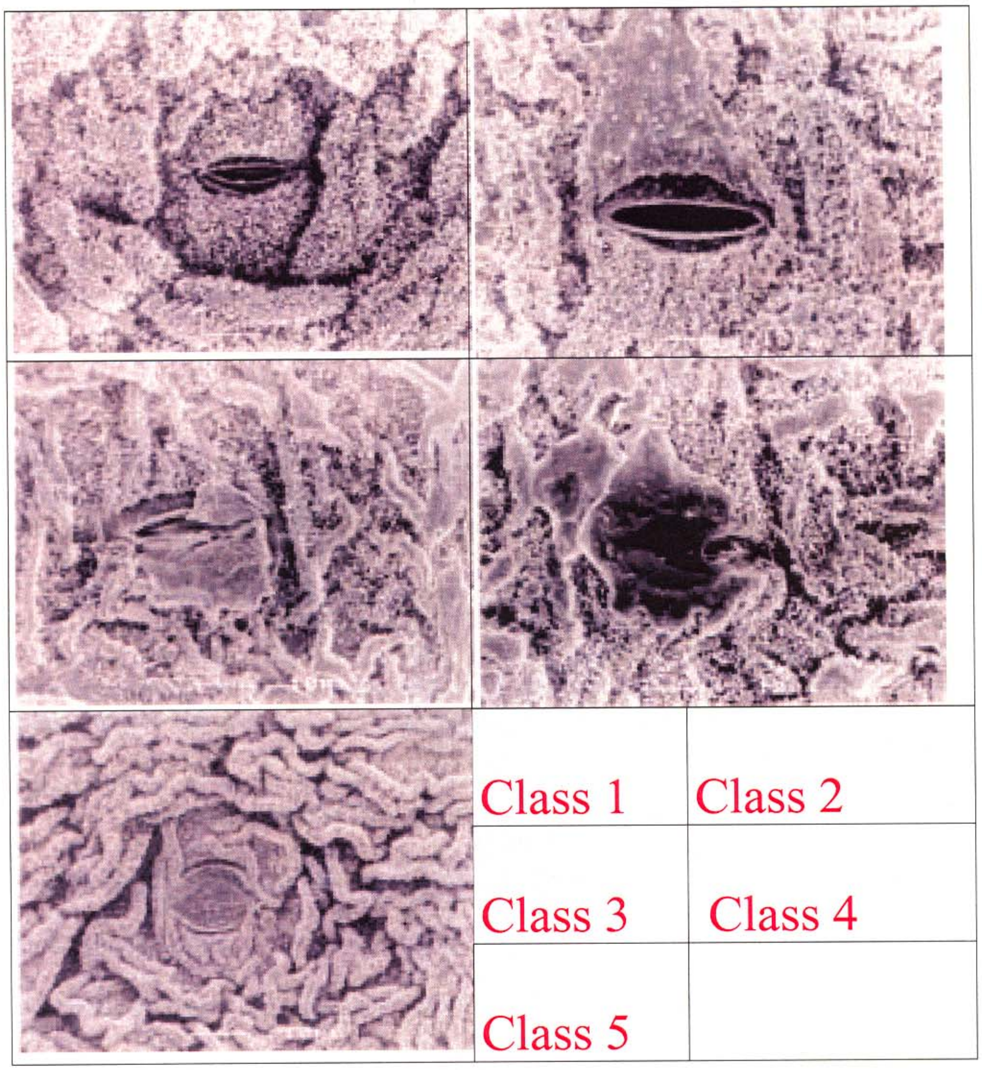

Fig. 1. Classification of changes of the epicuticular waxes of *Populus tremuloides.*

Materials and methods

Foliage of three trembling aspen (*Populus tremuloides* M i c h x.) clones differing in O_3 tolerance were examined during three growing seasons (1998–2000). Stomatal frequency was examined in year 2000. The clones were growing at three locations in a "natural" O_3 gradient from Rhinelander, northern Wisconsin (background O_3, 1996 SUM00 = 41.0 ppmh), to Kenosha, southern Wisconsin (high O_3, 1996 SUM00 = 70.4 ppmh) to Kalamazoo, southern Michigan (low O_3, 1996 SUM00 = 47.3 ppmh) (Karnosky et al., 1998a). The Rhinelander

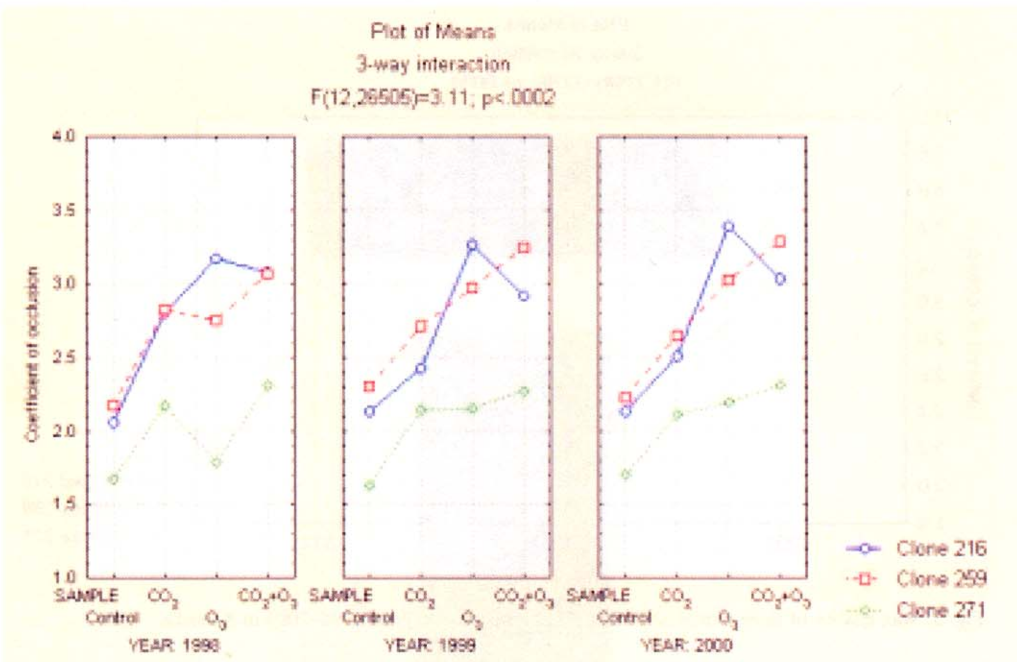

Fig. 2. Wax quality of clones 216, 259 and 271 of *Populus tremuloides* between 1998 and 2000 in Rhinelander (four treatmens).

locality encompassed 32 ha of land with twelve diameter treatment ring spaced 100m apart within a deer-fenced area. The rings were composed of 3 control rings, 3 rings with elevated O_3, 3 rings with elevated CO_2, and 3 rings with elevated O_3+ CO_2 (Dickson et al., 2000).

Air-dried leaves were treated by JEOL Ionsputtering prior to observation. They were assessed by scanning microscope JEOL 840 A. The wax surface SEM was done at the Canadian Forest Service's Fredericton, New Brunswick and at the Forest Research Institute, Zvolen. The wax quality was determined by evaluation of two hundred stomata per leave. Two leaves were evaluated per clone and month per site. Quantification changes (Table 1, Fig. 1) in the epistomatal wax structure of five distinct classes were defined by two criteria: different crystal wax morphology and the varying degree of changed wax structures to the stomata area (Maňkovská, 1996; Trimbacher, Eckmüllner, 1997). We used C_0 -Coefficient of occlusion (arithmetical mean of wax quality of 200 stomata per leave). The vegetation samples were evaluated by Kolmogorov-Smirnov statistical non-parametric test for qualitative attribute.

Results and discussion

Arithmerical means of wax quality (WQ) for individual clones of *Populus tremuloides* for Rhinelander in 1998–2000 is shown in Fig. 2. The worst wax quality was found for the most sensitive clone 259 whereas WQ was highest for the treatment with $O_3 + CO_2$. Quality of waxes for clone 216 was better than for clone 259, except for the treatment O_3, where WQ

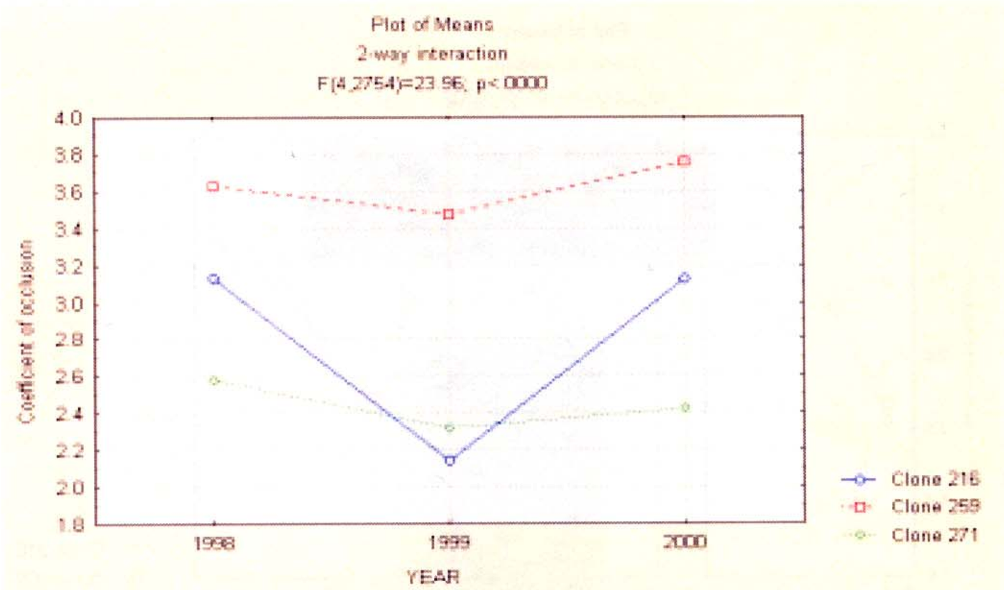

Fig. 3. Wax quality of three clones (216, 259,271) between three years 1998–2000 in Kenosha.

T a b l e 2. Evaluation of stomata frequency according to individual clones

Treatment	No. of stomata [cm^2] X (SD)		
Clone	216	259	271
Rhinelander			
Control	2779 (192)	3613(1472)	4599 (1437)
CO_2	3216 (509)	2245(1201)	6953 (579)
O_3	2833 (250)	5201 (710)	8390 (616)
O_3	3066 (475)	6377 (1180)	5748 (226)
Kenosha	3695	1027	6200
Statistical evaluation			
Treatment[a]	Insignificant		
Clones	Insignificant		
Treatment × clones	insignificant		
Locality [b]	Insignificant		
Clones	Insignificant		
Locality × Clones	insignificant		

Note:[a] results of analysis of variance for Rhinelander, we test influence of treatments O_3, CO_2, $O_3 + CO_2$ and clones 216, 259, 271 on No (number of stomata); [b] results of analysis of variance, we test influence of localities Rhinelander (we calculate only with variant control, Kenosha and clones 216, 259, 271 on No (number of stomata); values in table are means (SE -standard error of mean). ANOVA, *P < 0.05, **P < 0.01, ***P < 0.001. Means are designed different letters are different (P < 0.05, Turkey's multiple range test)

T a b l e 3. Analysis of variance for changes of epistomatal waxes of *Populus tremuloides* (three clones) in Rhinelander (four treatments) between 1998–2000

	Degree of freedom	Variance	F–test	Level of significance	Sign.
Treatment	3	0.6830	74.88	99.99	***
Years	1	0.0010	0.08	22.50	N
Clones	2	1.1490	125.91	99.99	***
Treatment × Years	3	0.0330	3.57	91.35	N
Treatment × Clones	6	0.0230	2.46	85.21	N
Years × Clones	2	0.0310	3.36	89.53	N
Residual	6	0.0090			
Together	23	0.2000			

Note: ANOVA, *P < 0.05, **P < 0.01, ***P < 0.001.

in all observed years was higher than WQ for clone 259. The best quality of waxes was found for clone 271, whereas the highest value of WQ was found for the treatment O_3 + CO_2.

Arithmetical mean of wax quality WQ for individual clones of *Populus tremuloides* for Kenosha in 1998–2000 is in Fig. 3. The most damaged waxes were found for clone 259; the least affected were for clone 271. WQ values were always higher in Kenosha than in Rhinelander.

Arithmetical mean of WQ for individual clones of aspen for Rhinelander, Kenosha and Kalamazoo in June, July, and August in 1999 is in Fig. 4. We found statistically significant differences in quality of stomata between three localities, three aspen clones, three months and four treatments in the localities when comparing results of quality of stomata (Table 4).

We found statistically significant differences in quality of stomata between three aspen clones and four treatments in the Rhinelander locality when comparing results of quality of

T a b l e 4. Variance analysis for changes of epistomatal waxes in *Populus tremuloides* (three clones) in Rhinelander, Kenosha and Kalamazoo between sampling periods

	Degree of freedom	Variance	F–test	Sign.
Localities	2	1.4040	236.60	***
Clones	2	1.9970	336.56	***
Month	2	0.1850	31.13	***
Localities Clones	4	0.1680	28.38	***
Localities × Month	4	0.0330	5.47	*
Clones × Month	4	0.0320	5.37	*
Residual	8	0.0060		
Together	26	0.3100		

Note: ANOVA, *P < 0.05, **P < 0.01, ***P < 0.001.

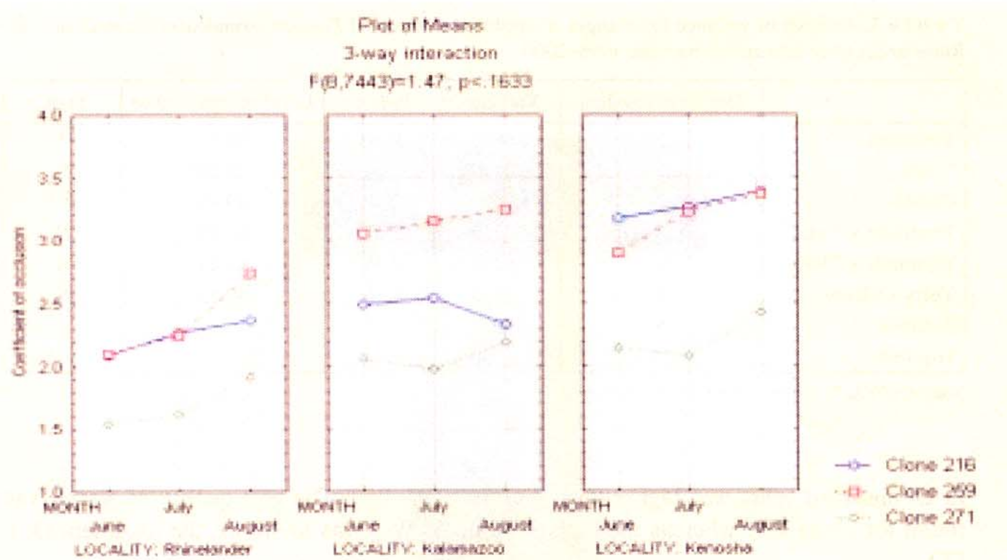

Fig. 4. Wax quality of three clones of *Populus tremuloides* between different sampling period and localities (Rhinelander, Kalamazoo and Kenosha).

stomata. Statistically significant differences were found between years, between years and treatments, between treatments and clones, and between years and clones. When comparing results we have found the largest differences in quality of stoma between individual

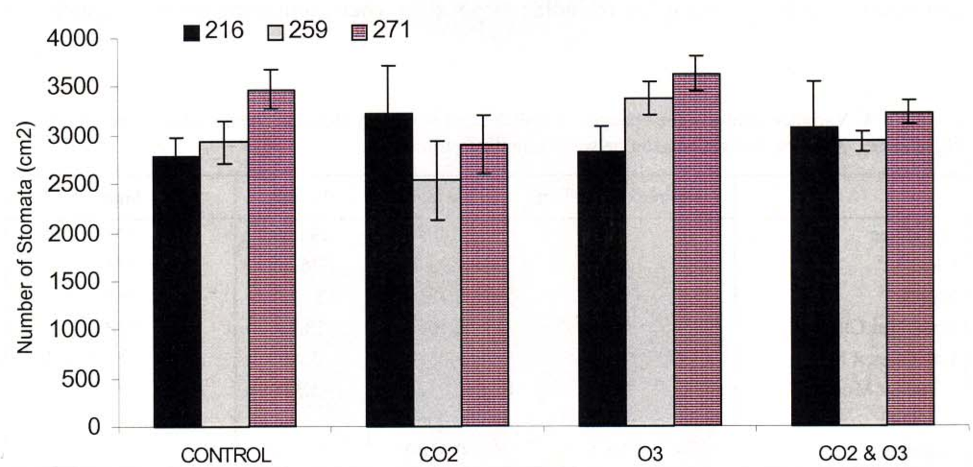

Fig. 5. Evaluation of stomata frequency according to individual aspen clones of in Rhinelander.

aspen clones at the Kenosha locality. Tolerant clone 271, unlike 216 and 259 had most relatively undamaged stoma in class 1 and 2. Clone 259 had most damaged stoma in class 4 and 5. When comparing quality of stoma from Kenosha with locality Rhinelander there has been found statistically significant difference between individual aspen clones with exception of clone 216 ($CO_2 + O_3$).

In the Table 2 and Fig. 5 we show the number of stomata according to particular aspen clones. We did not found any statistically significant differences in the frequency of stomata.

Translated by the authors

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Received 18.9.2002