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Impacts of greenhouse gases on epicuticular waxes of *Populus tremuloides* Michx.: Results from an open-air exposure and a natural O_3 gradient

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Structure of epicuticular waxes indicated phytotoxic effects of greenhouse gases on Populus tremuloides Michx.

Abstract

Epicuticular waxes of three trembling aspen (*Populus tremuloides* Michx.) clones differing in O_3 tolerance were examined over six growing seasons (1998–2003) at three bioindicator sites 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, 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. We recorded statistically significant differences between aspen clones and between sampling period (spring, summer, fall). We found no statistically significant differences between treatments or aspen clones in stomatal frequency.

Keywords: *Populus tremuloides* Michx; O_3 tolerance; Epicuticular wax; Stomatal frequency

1. 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 pollutant 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 (Dickson et al., 2000; 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), delays foliar senescence in autumn and stimulates aboveground

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(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 defense compounds and decreased concentration of antioxidants (Lindroth et al., 1993, 1997).

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, accelerate leaf senescence (Karnosky et al., 1996), decrease photosynthesis (Coleman et al., 1995a), alter carbon allocation (Coleman et al., 1995b, 1996), alter epicuticular wax structure and composition

Table 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 10–25% 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

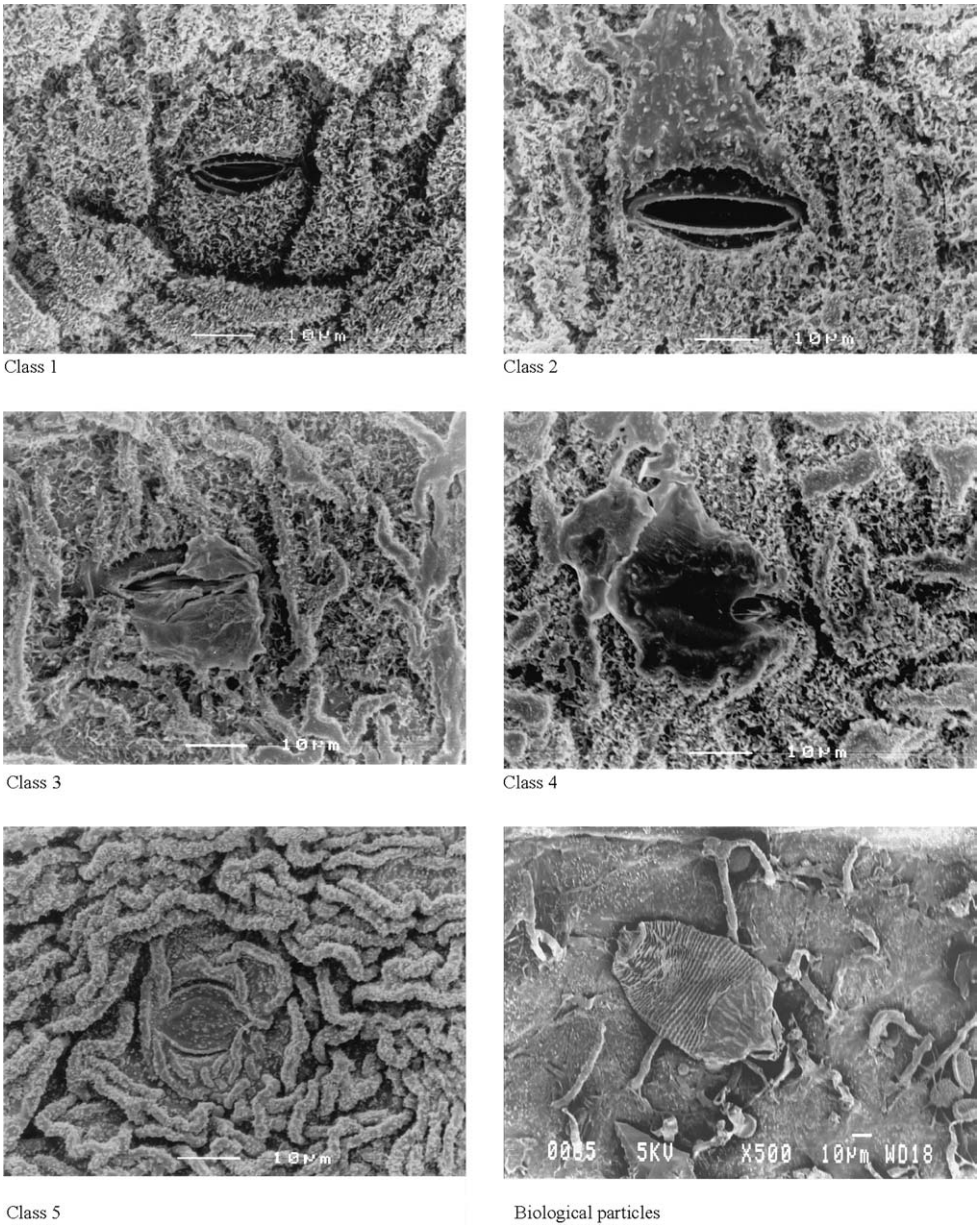

Fig. 1. Micrographs of trembling aspen stomata.