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ÉCLAIRE

Effects of Climate Change on Air Pollution Impacts and Response Strategies for European Ecosystems

Seventh Framework Programme

Theme: Environment

D 11.5 Quantification of minimum epidermal conductance under different loads of particles

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PU	Public	☒
PP	Restricted to other programme participants (including the Commission Services)	☐
RE	Restricted to a group specified by the consortium (including the Commission Services)	☐
CO	Confidential, only for members of the consortium (including the Commission Services)	☐

1. Executive Summary

The minimum epidermal conductance (g_{min}) is the conductance to plant water diffusion across the foliar epidermis once stomata are closed, i.e., through the cuticle and any leaky stomata (e.g. Shepherd, 2006; Takamatsu et al., 2001). The g_{min} varies many-fold across species, and can be a potentially strong determinant of drought tolerance, since a lower g_{min} better enables maintenance of hydration. g_{min} is thus an ecologically important parameter, which e.g. may determine the altitudinal limit of trees (Baig and Tranquilini, 1980; Grace, 1990). While the cuticular part of g_{min} is determined by physiological factors like wax thickness and wax microstructure (Sheppard, 2006), the development of leaky stomata has remained elusive, and could be controlled either by physiology or by environmental factors including air pollution (Marks and Lechowicz, 2007). The contribution of leaky stomata to g_{min} can be large or even dominant.

A possible mechanism for the development of leaky stomata is the hydraulic activation of stomata or HAS (Burkhardt, 2010). This concept describes the development of thin layers of liquid water along inner stomatal walls which eventually form a liquid water connection between the interior of the leaf and the leaf surface. Such connections may then act similar to wicks, increasing transpiration. They develop from hygroscopic substances on the leaf surface (usually deposited aerosols) which form highly concentrated solutions, often close to saturation. The water surface tension of such concentrated solutions is determined by the ion species, and specifically ‘chaotropic’ ions (e.g., nitrate, iodide) can considerably lower surface tension, which promotes HAS (Burkhardt et al., 2012). According to this hypothesis, salts on the leaf surface should lead to an increase of g_{min}. So far, systematic investigations on this topic do not exist and the approach taken here was the spraying of beech and pine seedlings with different types of salts, with or without surfactants. Surfactants were added because a mechanism similar to HAS had been proposed earlier to explain the decline of coastal forests in Italy, where sea salt polluted with surfactants from nearby landfills was deposited to the leaves (Grieve et al., 1978, Rettori et al., 2005).

Seedlings of pine (*Pinus sylvestris*) and beech (*Fagus sylvatica*) were sprayed by a standardized technique with solutions of NaCl, $NaNO_3$, $(NH_4)_2SO_4$, KI (25 or 50 mmol l^{-1}), with or without surfactant, respectively. Samples were taken 6 – 7 weeks after spraying and g_{min} was then measured by the weight loss of excised leaves under controlled conditions (leaf drying curves). g_{min} was also measured for seedlings grown in greenhouses with filtered, almost particle free air.

All salts caused higher g_{min}, with increases between 18% and 34% compared to the control. The addition of surfactant caused additional increases for sodium chloride and ammonium sulphate, but not for sodium nitrate. The highest overall increase of g_{min} was 96% , measured for NaCl+surfactant when sprayed on pine seedlings. The exclusion of ambient particles lowered g_{min}. of beech by 10%.

These results show that salt particles on leaf surfaces have an impact on plant water conditions and support the hypothesis of stomatal liquid water loss by HAS. Although only pure salts were used in this experiment, lower g_{min} in filtered air supports the idea that the same mechanism is also generally valid for many ambient particles deposited to leaves.

Given the importance of g_{min} for drought tolerance and in ecology, these findings may be significant. Air pollution (by accumulation of hygroscopic particles on leaves) and drought effects on plants may be directly linked by this mechanism. Although not considered in Central European forest decline, a potential impact of the mechanism is supported by the recent finding that leaf surface particles are capable to produce the “wax degradation” phenomenon (Burkhardt & Pariyar, 2013). The regional forest decline recently observed in the western USA and throughout the world (van Mantgem et al., 2009; Allen et al., 2010; Anderegg et al., 2013) is connected to increasing drought periods due to climatic change. Deposited particles from air pollution might aggravate these drought effects.

Objectives:

The main objective was to show and quantify the impact of deposited salt particles on minimum epidermal conductance g_{min}.
An increase of g_{min} by particles would support the hypothesis that particle deposition and hydraulically activated stomata (HAS) are important and so far neglected factors in plant transpiration.
A differentiated influence of different salts was predicted. Solutions with low surface tension (chaotropic ions or salts together with surfactants) should have the highest impact.
Contrary to a g_{min} increase by amendment of particles to leaf surfaces, the exclusion of particles should lead to a decrease of g_{min} .

2. Activities:

Scots pine (*Pinus sylvestris* L.) saplings (1+2 yrs, Schlegel & Co. Gartenprodukte GmbH, Riedlingen, Germany) were grown in well watered soil (Type: “Nursery & Perennial” H. Nitsch & Sohn GmbH & Co.KG, Kreuztal-Eichen, Germany). European beech (*Fagus sylvatic*a L.) saplings (2 yrs) were grown in well watered soil (Type: “Pot” with 20% sand mixture). The trees were sprayed by an automated spraying system (“Phytron Electronic”, developed by Agricultural Technology Institute, University of Bonn). The whole plants were sprayed with solution droplets having volumes of 0.5 - 5 µl until water holding capacity was exceeded, i.e. water had started dripping from the needles. Application to the soil was avoided by covering the pot and the first internodes header shoot by a thin plastic bag during spraying.
The Scots pine, was sprayed with 50 mM solutions of hygroscopic salts NaCl, $NaNO_3$, $(NH_4)_2SO_4$), and KI. NaCl was also sprayed with a surfactant (RSO5) 1g/L, a rapeseed ethoxylate acting as a surfactant; courtesy: Cognis AgroSolutions, Germany)). The beech plants were sprayed with 25 mM $(NH_4)_2SO_4$, and $NaNO_3$, with and without surfactant. Spraying with deionised water was used as a control for the salt solution treatments. The water holding capacity of pine needles was assumed to be 100 gm^{-2} leaf area, which corresponds with a continuous spray solution film of 0.1 mm (Trautner and Eiden, 1988). After drying of the 50 mM salt solution droplets, a hypothetical homogeneous particle loading of approximately 30-85 µg cm^{-2} remained, depending on the molecular weight of the respective salt. These are high but not completely unrealistic values compared to reported particle loading (Burkhardt, 2010; references therein, Saebo et al., 2012).
After the salt application, the treated plants stayed in the controlled greenhouse. g_{min} measurements (3 replicate plants) were done 48 days after treatment. The treated plants were well-watered a day before the samples were taken to rehydrate the shoots overnight. The samples were taken from the current year needles (pine) from the middle part of the auxiliary shoot of the same internodes in the next morning. These current year shoots had developed and grown in the experimental conditions. A pair of needles was harvested from each treated plant very carefully with the help of a scalpel, detaching the same aged needles at the very basal part and keeping the leaf-sheath still covering the needles. Smooth surface plastic forceps was used to handle the samples and the needles were handled with care. Beech leaves were taken from the side branch of the main header shoot at the middle part of the canopy, which were fully developed and healthy. A sample was taken from each plant. No physical injury was visible due to handling. Paraffin wax was used to seal the leaf/needle basis. The maintenance of humidity and temperature was regulated in the drying environment. Each sample contained a pair of needles or a leaf. The samples were labelled and leaf area was measured with a leaf area measuring system, based on a commercial scanner with specifically adapted software (OMA, HGo Tech, Bonn, Germany). The leaves/needles were attached to a lab tape on a stick and then hung for at least one hour into a ventilated, dark drying chamber to dehydrate them. They were then taken from the tape and weighed on a digital micro balance. This time was denoted as time 0. Then the samples were again taped back onto the stick and placed back into the drying chamber. The second weighing was done at least 20 minutes for beech leaves and 3 hours for pine needles after the previous weighing. This process was repeated for about a day for beech and 3 days for pine needle with 8-10 measurements. Then, the leaf area was again measured. Temperature and RH of the drying conditions were continuously recorded every 15

min by a data logger. The Arden Buck equation (1996, Buck Research CR-1A User´s Manual) was used to calculate the saturated vapour pressure (VPsat, kPa). The leaf drying measurements, relative humdity, temperature and leaf area data were transferred to a g_{min} analysis spreadsheet tool (Sack and Scoffoni, 2011). Mean g_{min} values based on intervals were calculated by selecting the linear points (at least 4 points) on the graphs, which was also close to the value calculated by the slope in the graph. The g_{min} values were then statistically compared. Analysis was performed using one way analysis of variance. As the results of the unsealed needles were similar for both dates, data from the first and the second spraying were pooled for statistical analysis.
The multiple pair wise comparison versus control group was performed using Holm-Sidak method. Significantly different values are indicated by $P < 0.05$ (*, or different lowercase letters), $P < 0.01$ (**) and $P < 0.001$ (***).

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3. Results:

The g_{min} was generally higher for beech leaves than for pine needles (Fig. 1). For both, beech and pine, g_{min} increased by spraying with all salt solutions, compared to the control. For pine needles (Fig. 1a), the increase was 18% (sodium nitrate), 34% (ammonium sulphate and sodium chloride), and 45 % (potassium iodide). When sodium chloride was combined with RSO5 surfactant, the overall increase was 96%. For beech leaves (Fig. 1b), the increase was 21% (ammonium sulphate) and 28% (sodium nitrate). In combination with RSO5 surfactant, the overall increase was 34% (ammonium sulphate) and 26% (sodium nitrate).
A clear order of transpiration increase according to the position in the Hofmeister series (‘chaotropic’ and ‘kosmotropic’ salts) could not be established.
The exclusion of ambient particles resulted in a lower mean g_{min} of beech by 10% compared to ambient air, although this result was not significant.

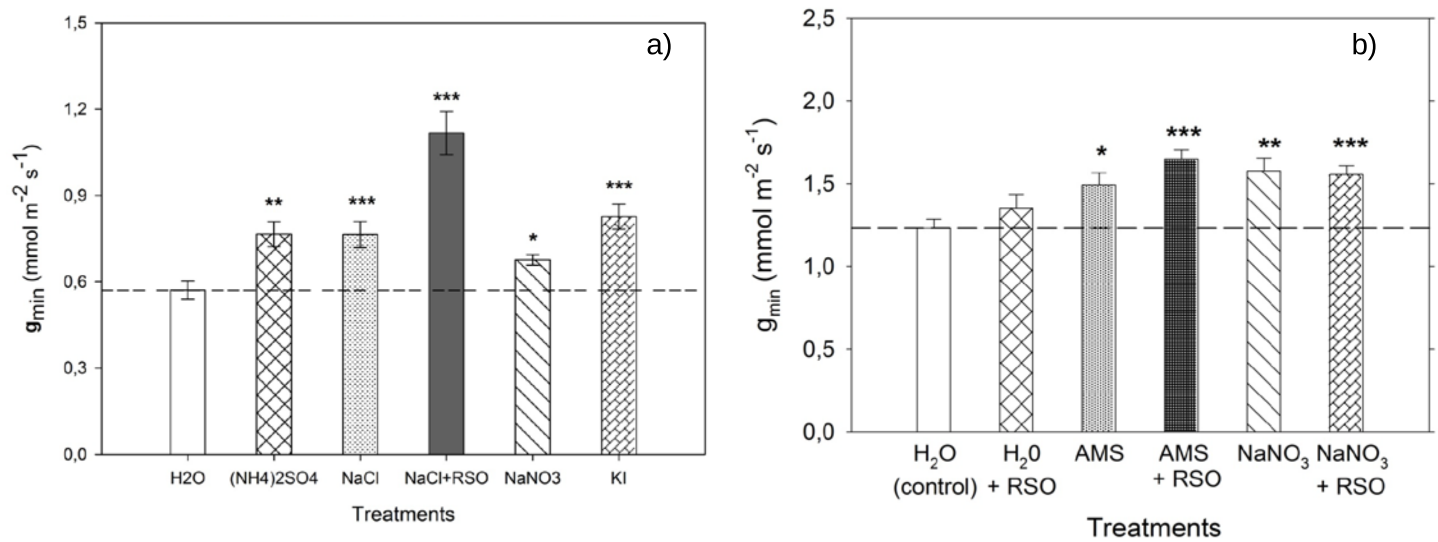

Fig. 1: Epidermal minimum conductance of a) pine needles and b) beech leaves after treatments with different salt solutions. AMS: ammonium sulphate. RSO: rape seed ethoxylate surfactant.

4. Milestones achieved:

MS 50 (Submission of data to database for modelling purposes) has been partially fulfilled, as the presently available data were submitted to ECLAIRE database.

5. Deviations and reasons:

none

6. Publications:

Burkhardt J., Pariyar S. (2013): Particulate pollutants are capable to 'degrade' epicuticular waxes and to decrease the drought tolerance of Scots pine (Pinus sylvestris L.). E*nvironmental Pollution, doi: 10.1016/j.envpol.2013.04.041*

7. Meetings:

Results from the project were presented at the following international meetings:

IUFRO RG 7.01 International Conference 2012 'Biological Reactions of Forests to Climate Change and Air Pollution', Kaunas, Lithuania, 18-27 May, 2012. J. Burkhardt: Direct interactions of hygroscopic particles with leaf/needle surfaces and plant water relations (oral presentation).

International Workshop and Meeting of the German Society of Plant Nutrition, Bonn, Germany, 5-7 September, 2012. S. Pariyar: Effects of ambient aerosol particles on the water relations of crop plants (oral presentation).

ECLAIRE 2nd GA, Edinburgh, UK, 15-18 October. S. Pariyar: Effects of aerosol deposition on stomatal functions (oral presentation).

Biohydrology Conference 2013, Landau, Germany, 21-24 May, 2013. J. Burkhardt: From leaf wetness to deliquescent leaf surface particles – microscopic water at the plant/atmosphere interface (oral presentation).

International Plant Nutrition Colloquium, Istanbul, Turkey, 19-22 August 2013. S. Pariyar: Aerosol particles affect the water economy of plants (poster presentation).

Goldschmidt 2013, Florence, Italy, 26-30 August, 2013. J. Burkhardt: Aerosols and plant leaf surfaces (oral presentation).

Deutsche Botanikertagung 2013, Tübingen, Germany, 30 Sept – 4 Oct 2013. J. Burkhardt: Hygroscopic leaf surface particles reduce the drought tolerance of Scots pine by deliquescence, stomatal penetration and the establishment of wick-like structures (accepted for oral presentation).

8. List of Documents/Annexes:

Publication Burkhardt and Pariyar, 2013, Environmental Pollution

Press release, University of Bonn, June 2013