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New Initiatives

Harmful Effects of Atmospheric Nitrous acid on the Physiological Status of Scots Pine Trees

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Capsule

Exposure to HONO over 2 months affects photosynthesis and nutrient status of pine trees.

Abstract:

An open top chamber experiment was carried out in the summer of 2003 to examine the effect of nitrous acid (HONO) gas on the physiological status of Scots pine saplings (*Pinus sylvestris*). 4-year old pine trees were exposed to two different levels of HONO gas (at *ca.* 2.5ppb and 5.0ppb) and a control (filtered air) from early evening to early morning (18:00~6:00), in duplicate open-top chambers. Significant decreases in the ratios of chlorophylls *a* to *b*, an increase in the carbon to nitrogen (C/N) ratio, and a reduction of maximum quantum yield of PS II (*Fv/Fm*) in pine needles were observed after the 2 months fumigation. Cation contents of pine needles were also decreased by the fumigation with HONO gas. The results could be explained by the harmful effects of

OH radicals, generated from photolysis of HONO gas, and/or aqueous phase HONO (NO_2^-/HONO), on the photosynthetic capacity of pine needles.

1. Introduction

Nitrous acid (HONO) in the atmosphere is generated from heterogeneous reactions of nitrogen dioxide (NO_2) and water on ground surfaces such as soil, walls of buildings and airborne particle surfaces($2NO_2 + H_2O \xrightarrow{surface} HONO + HNO_3$), and as direct emissions from automobile exhausts (Lammel and Cape, 1996). Photolysis of HONO gas ($HONO+hv \rightarrow \cdot OH+NO$) is a source of hydroxyl radical (·OH), the most important oxidant in the troposphere. Concentrations of HONO gas in ambient air are reported to be 0.1-10 ppb (parts in 10^9 by volume) in urban areas and 0.01-1 ppb in rural and remote areas (Lammel and Cape, 1996). Night-time concentrations of up to 5 ppb (Zhang et al., 2003) have been reported, with mean values of 2 ppb and maxima of over 8 ppb in urban areas (Bari et al., 2003). Due to its rapid decomposition by sunlight, HONO gas is present at higher concentrations at night, with lower but not zero concentrations in daytime. Uptake of HONO gas by higher plants is proportional to HONO concentrations and linearly related to stomatal conductance, limited by diffusion of HONO through stomata (Schimang et al., 2006). However, effects of HONO gas on higher plants are not yet known because long term fumigation experiments with HONO gas on higher plants have not been conducted; the high instability of HONO gas to solar radiation largely inhibits the determination of any cause-effect relationship from field studies. This experiment examined the effects of HONO gas on Scots pine trees using open top chambers for fumigation.

2. Methodology

2.1. Chamber experiments

Open top chambers (OTCs: 3m height and 3m diameter, with glass walls and with the top open to the atmosphere), situated at the field experimental facilities at the Edinburgh Research Station, CEH, were used for fumigating 4-year old Scots pine trees (*Pinus sylvestris* L.) with HONO. Each of 6 chambers contained 6 Scots pine saplings in pots in a peat:loam:grit (3:2:1 by volume) mixture, regularly watered, and exposed to ambient rainfall. Ambient air, pre-treated with charcoal filters, was introduced into the OTCs from an internal perforated polyethylene torus mounted above the plants at a flow rate of *ca.*14 m^3 min^{-1}. Fumigations of 0, *ca.*2.5 or *ca.*5.0 ppb HONO gas were carried out from 18:00 to 6:00 every day during two months (July–September) in 2003. Sunrise and sunset times during the experimental period were 3-5 am and 7-9 pm, respectively, at the site and thus the fumigation was conducted not only at night but also at low light intensity periods at dusk and dawn. During the exposure experiment, average air temperature (24 h), solar radiation at noon, and total precipitation amount were 13.1℃, 0.45 kW m^{-2}, and 194 mm. Measurements of the photolysis rate of NO_2 (jNO_2) inside and outside the OTCs during the exposure experiment indicated that light intensity in the chamber (at jNO_2 wavelengths) was on average 33% of outdoors; transmission of total solar radiation, as measured by a Kipp-type solarimeter, was 85%.

2.2. Preparation of HONO gas

HONO gas was generated by a method similar to that of Taira and Kanda (1990). Dilute solutions of sodium nitrite and sulphuric acid were separately pumped by a peristaltic pump at a flow rate of *ca* 0.1ml min^{-1} into the two side ports of a 4-port reaction tube (*ca* 1.0 ml internal volume) where they were mixed together. By changing the sulphuric acid concentrations, *ca.*2.5 and *ca.*5.0 ppb of HONO gas (with ±10%

variation of the generation rate) were produced in the generation system. Ambient air, passed through charcoal and silica gel columns using an air pump, was used for carrier gas at a flow rate of 0.4-0.5 l min^{-1}, which entered the reaction tube by the bottom port. HONO vapour was transferred to the carrier air flow during passage through a stripping coil (4mm i.d. x 25mm coil diameter x 60mm coil length) while waste solution was pumped out by the peristaltic pump from the end of the stripping coil. The HONO gas generated in the stripping coil was introduced into the main air supply of the OTCs through PTFE tubing, which was completely encased in black plastic tubing to prevent HONO photolysis. HNO_3, NO and NO_2 can be by-products of HONO generation in such a system; the higher HONO treatment (5 ppb) was associated with a concentration of NO around 25 ppb, but negligible amounts of NO_2 and HNO_3 were detected (see below).

2.3. Analytical methods

Total NOx concentrations were monitored continuously in one fumigated chamber by a chemiluminescent NOx monitor (Monitor Labs 8840), and air in all chambers was sampled regularly using a cylindrical denuder coated in Na_2CO_3 and analysed by ion chromatography (Metrohm) for NO_2^- and NO_3^-. HONO gas concentrations in the chambers were calculated from the NO_2^- concentrations and volume of air passed through the denuder. Measured HONO concentrations during fumigation were 2.3 (± 0.4) and 4.7 (± 0.5) ppb for the two treatments; the uncertainty is the range of the duplicate chambers. Nitrate concentrations (from HNO_3) were less than 15% of the measured nitrite concentrations (from HONO). Any contribution from NO_2 to nitrite concentrations would have been small because of the low concentrations of NO_2 (<10 ppb, not related to the fumigation treatment).

The maximum quantum yield of PS II (*Fv/Fm*) was evaluated by measuring chlorophyll fluorescence using a portable chlorophyll fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich), with 4 clips per tree and 12 min dark adaptation. The C and N contents of needles were measured by an elemental analyzer (CHN-2000II, Perkin Elmer) after freeze-drying the needles. Chlorophyll contents were determined by colorimetry at 720 nm (blank), 664 and 647 nm after solvent extraction (0.1g fresh wt needles in 3 ml dimethylformamide for 4 days at room temperature). Cation contents were analyzed by inductively coupled plasma atomic emission spectrometry (Optima3000, Perkin Elmer) after needle samples were pre-treated with chloroform for 2 min to remove surface deposits then digested in a microwave system with HNO_3. All measurements were made on current-year needles, sampled at the end of the experiment (September).

3. Results and Discussion

3.1. Overall results

After 2 months fumigation with HONO gas, a significant decrease of photosynthetic capacity was detected from the decreased chlorophyll *a* content and maximum quantum yield of PS II (*Fv/Fm*) in needles (Table 1), while the overall content of chlorophyll *a*+*b* was unchanged by the HONO fumigation and thus the ratio of chlorophyll *a* to chlorophyll *b* was significantly reduced. Moreover, considering the increased C/N ratios observed, it is likely that there was a reduction in the needle content of proteins such as Rubisco.

A decreasing trend in Ca, Na, Fe and Al concentrations in the pine needles with increasing HONO fumigation was observed, while K and Mg concentrations showed no trend. Results of the cation analysis indicate that: 1) supply of nutrients from roots may

be reduced due to damage to root system, and/or 2) cation leaching was increased from needles fumigated with HONO gas.

3.2. Mechanisms of damage – role of OH radicals

These results, especially the decreasing trend of the ratio of chlorophyll *a* to *b*, and PSII (*Fv/Fm*), indicate that the primary process of photosynthesis was damaged by HONO fumigation. The damage caused by HONO could be explained by harmful effects of OH radicals generated by sunlight irradiation of HONO. Even though the fumigation was mostly at night, to mimic the pattern of exposure seen in the atmosphere, even the low levels of UV irradiance through the glass walls of the OTCs at dawn and dusk would have generated OH radicals from gas-phase HONO, and also from HONO adsorbed on leaf surfaces or dissolved in surface humidity. Similar effects have been observed in experiments in which young Japanese red pine (*Pinus densiflora*) plants were exposed to OH radicals (Kobayashi et al., 2002; Nakatani, 2004; Chiwa et al., 2005).

In theory HONO might act as both a source of OH radical and a fertilizer for higher plants. Fertilizer effects of HONO on the physiological status of higher plants could be important because, after uptake of HONO gas through stomata, nitrite (the direct product of HONO in solution) could be quickly metabolized (Schimang et al., 2006). However, in this study there was no evidence of HONO gas acting as a fertilizer, because the C:N ratio increased, rather than any decrease that might have been expected if HONO (or trace contamination by HNO_3) had acted as a source of nutrient N.

Several factors could have been involved in determining the observed HONO effects on pine needles:

1) HONO gas and/or NO_2^-/HONO, deposited on plant surfaces, were quickly

photodecomposed into OH radical, or reacted with water and HNO_3 on the surfaces of plants and chamber walls, and thus any fertilizer effects of HONO were negligible;

2) Stomatal closure at night, during our fumigation, protected plants from the entry of NO_2^-/HONO into the mesophyll air space and thus minimized the fertilizer effects of NO_2^-/HONO; or

3) Even if a portion of HONO gas was deposited on the needle surfaces and penetrated through stomata during daytime, the fertilizer effects of NO_2^-/HONO were overwhelmed by harmful effects of OH radicals. Acidified needle surface wetness could have played an important role in the photo-generation of OH radicals and also in the process causing damage to pine needles.

4. Conclusions

As far as the authors are aware this is the first study that shows the harmful effects of atmospheric HONO on higher plants at concentrations that are present in polluted air. The average exposure concentrations over the whole period (fumigation for 12 h d^{-1}) were close to 1.25 and 2.5 ppb, which are within the range of average measured urban values. Although plants were exposed simultaneously to up to 25 ppb NO during fumigation, this would be usual for conditions in which HONO concentrations of a few ppb are found, i.e. in urban areas at night. The two-month average concentration of NO (day and night) was around half the fumigation value (12 h d^{-1}), and below any concentration expected to have an effect on plants (WHO, 1997).

Atmospheric OH radicals may be not only the “vacuum cleaner” for cleaning the atmosphere but may also be detrimental to plant life. Studies on the mechanisms of HONO effects and fumigations of various higher plants are now required.

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Table 1 Results of measurements on current-year needles at end of experiment. Values shown are means for each treatment (± range of mean of 6 plants for each duplicate chamber). Statistically significant effects of treatment are shown in bold (ANOVA: P<0.05); where the linear relationship between treatment concentration and response is significant (linear regression: P<0.05) the data are shown in *bold italics*.

Measurement	**Control**	**(HONO) 2.5 ppb**	**(HONO) 5 ppb**	**Probability ANOVA/*linear***
Chlorophyll *a* µg g^{-1} fresh wt	309 ± 34	250 ± 2	228 ± 23	*0.059*
Chlorophyll *b* µg g^{-1} fresh wt	161 ± 42	241 ± 44	237 ± 6	*0.20*
Chlorophyll *a+b* µg g^{-1} fresh wt	470 ± 76	490 ± 42	465 ± 29	*0.94*
Chlorophyll *a/b*	**2.00 ± 0.32**	**1.08 ± 0.20**	**0.96 ± 0.07**	***0.044***
Fv/Fm	**0.731± 0.001**	**0.678 ± 0.009**	**0.688 ± 0.001**	**0.037**
C:N ratio	**48±1.9**	**50±2.4**	**54±0.3**	***0.047***
Tissue N mg g^{-1} dry wt.	10.4±0.48	10.0±0.53	9.3±0.07	*0.076*
K mg g^{-1} dry wt	7.2 ± 0.5	6.6 ± 0.6	7.3 ± 0.1	*0.84*
Ca mg g^{-1} dry wt	**2.1 ± 0.1**	**1.8 ± 0.06**	**1.9 ± 0.02**	**0.034** *0.18*
Mg mg g^{-1} dry wt	1.11 ± 0.16	1.13 ± 0.05	0.94 ± 0.04	*0.38*
Na µg g^{-1} dry wt	**23 ± 2**	**19 ± 2**	**14 ± 1**	**0.023** ***0.018***
Fe µg g^{-1} dry wt	**34 ± 0.6**	**29 ± 1**	**27 ± 0.2**	**0.024** ***0.009***
Al µg g^{-1} dry wt	**64 ± 14**	**47 ± 8**	**34 ± 5**	**0.026** *0.07*