Submicron ammonium sulfate particles deposited on leaf surfaces of a leafy vegetable (Komatsuna, *Brassica rapa* L. var. *perviridis*) are taken up by leaf and enhance nocturnal leaf conductance

Akira Motai, Marina Yamazaki, Nana Muramatsu, Makoto Watanabe, Takeshi Izuta

A simulation using a chemical transport model predicted that proportionality of sulfate (SO$_4^{2−}$) is a dominant component of inorganic aerosol, and are regarded as a long-range transboundary air pollutant in Asia. However, only a few studies have focused on the effects of submicron AS particles on plants. In the present study, we investigated the amount of submicron AS particles deposited on the leaf surface of komatsuna plants (*Brassica rapa* L. var. *perviridis*), and their effects on nocturnal leaf ($g_{n_d}$) and cuticular ($g_{w_c}$) conductance. The plants were grown for 23 days after sowing in a naturally lit phytotron at air temperature of 25.0 ± 1.0°C/18.0 ± 1.0°C (6:00-18:00/18:00-6:00) and relative air humidity of 70 ± 5%, and were artificially exposed to submicron AS particles at the 21st day after sowing. The exposure to submicron AS particles resulted in average daily atmospheric concentration of SO$_4^{2−}$ in PM$_{2.5}$ inside the phytotron of 16.5 μg m$^{-3}$. After the end of exposure, the plants were kept under light or dark conditions for 48 h in the phytotron. The amount of SO$_4^{2−}$ deposited on the leaf surface reduced over time, but did not significantly differ between the plants kept under light and dark conditions at 0, 6, 12, 18, 30, and 48 h after the end of exposure. An exposure to submicron AS particles with average daily atmospheric concentration of SO$_4^{2−}$ in PM$_{2.5}$ inside the phytotron of 24.7 μg m$^{-3}$ did not significantly affect the $g_{n_d}$ of the plants. At 1.5 and 5 h after the end of exposure, the $g_{n_d}$ was significantly higher in the submicron AS particle-exposed plants than in the non-exposed control plants. These results indicate that submicron AS particles deposited on the leaf surface enhance the nocturnal water consumption of komatsuna plants.

1. Introduction

Anthropogenic aerosols emitted by the combustion of fossil fuels mainly exist at the submicron level (Horvath, 2000). The atmospheric concentration of aerosols in Asia has been increasing because of the increased consumption of fossil fuels along with rapid economic growth (Ohara et al., 2007; Kurokawa et al., 2013; Yang et al., 2015; Saxena et al., 2017). In Asia, sulfate (SO$_4^{2−}$) is a dominant component of submicron anthropogenic aerosol, and generally exists in the form of ammonium sulfate [(NH$_4$)$_2$SO$_4$] (AS) or ammonium hydrogen sulfate (NH$_4$HSO$_4$) in the atmosphere (Kaneyasu et al., 2014; Sun et al., 2015; Hatakeyama, 2017). The atmospheric environment in Japan is affected by transboundary air pollutants such as aerosols from the Asian continent (Aikawa et al., 2010; Moreno et al., 2013; Kaneyasu et al., 2014). A simulation using a chemical transport model predicted that proportional contribution of non-sea-salt SO$_4^{2−}$ (nss-SO$_4^{2−}$) from China to the total average annual nss-SO$_4^{2−}$ concentration in Japan was 50–70% (Aikawa et al., 2010). Especially in the western part of Japan, relative contribution of PM$_{2.5}$ transported from the Asian continent to the concentration of PM$_{2.5}$ observed at the site was simulated to reach as much as 90% (Ikeda et al., 2014). These reports indicate that the atmospheric environment in Japan is relatively dominated by the inflow of air pollutants from the Asian continent and has been facing the problems of transboundary air pollution due to aerosols such as AS particles. Based on the facts concerning the atmospheric pollution induced by submicron aerosol particles in Asia, there is a scientific question whether submicron aerosol particles such as AS particles in the atmosphere have effects on crops and trees in Asia.

An increase in the atmospheric concentration of submicron aerosol particles alters the intensity of solar radiation reaching the land surface by scattering or absorbing solar radiation, and consequently may indirectly influence the productivity of plants (Stanhill and Cohen, 2001;
Niyogi et al., 2004; Carslaw et al., 2010; Kalidindi et al., 2015; Lu et al., 2017). In addition, aerosol particles deposited on the leaf surface can directly affect the growth and physiological functions of plants depending on the properties of the particles (Grantly et al., 2003; Burkhardt, 2010). Our previous study showed that the growth and yield of komatsuna plants (Brassica rapa L. var. perviridis), a popular leafy vegetable in Japan, were reduced by the exposure to submicron AS particles (Motai et al., 2017). At present, however, little is known about the direct effects of submicron AS particles on physiological functions of plants. To better understand the interaction between the atmospheric environment and plants in Asia, further studies on the direct effects of submicron aerosols on physiological functions of plants are required.

When hygroscopic materials such as AS particles are deposited on the leaf surface, the particles absorb water and deliquesce on the leaf surface (Burkhardt, 2010; Burkhardt et al., 2012). The deliquescent salt solution establishes a thin hydraulic film that connects the leaf surface to the interior via stomatal pores, a hypothesis known as the “hydraulic activation of stomata (HAS)” (Burkhardt, 2010; Burkhardt and Grantly, 2017). It has been considered that water flows from the leaf interior to the surface as long as the water potential of deliquesced salt solution on the leaf surface is lower than that of solution in leaf. Burkhardt et al. (2001) reported that hygroscopic NaNO3 particles enhanced water loss from leaves with a small stomatal aperture. Because stomata do not completely close during the nighttime (Snyder et al., 2003; Caird et al., 2007), deliquescent salt solution may enter the leaf interior along the wall of stomata and induce water flow from the leaf interior to the surface. Thus, it is possible that nocturnal stomatal conductance will be enhanced by the deposition of hygroscopic materials such as AS particles onto leaves. In addition, Burkhardt and Pariyar (2014) reported that spraying AS solution onto the needles of Scots pine (Pinus sylvestris L.) enhanced the minimum epidermal conductance (i.e., cuticular conductance). These reports suggested that submicron AS particles deposited on the leaf surface may enhance nocturnal leaf conductance (i.e., stomatal and cuticular conductance), leading to increased water loss from the leaf interior. However, no information is available about the effects of submicron AS particles on nocturnal leaf conductance to water vapor.

As described above, deposited AS particles seem to enhance water loss from the leaf. Thus, the retention time of the particles on the leaf surface may affect the degree of their effects on nocturnal leaf conductance. Our previous study showed that the amount of submicron AS particles deposited on leaf surface of komatsuna plants reduced over time (Motai et al., 2017). Therefore, the degree of the increase of nocturnal leaf conductance caused by AS particles should also decrease over time because of the reduced amount of AS particles deposited on the leaf surface. The aim of the present study was to clarify the effects of submicron AS particles on leaf conductance to water vapor in komatsuna plants (Brassica rapa L. var. perviridis). The reasons for using komatsuna plants as plant material in the present study are that this species is a representative leafy vegetable in Asian countries such as Japan and China, and leafy vegetable seems to be relatively susceptible to submicron AS particles because the fraction of leaf to the whole-plant (i.e., deposition area of the particles) in leafy vegetables is relatively large as compared to the other types of crops. In the present study, we investigated the amount of submicron AS particles deposited on the leaf surface of komatsuna plants, their reduction over time after the end of exposure to AS particles, and their effects on nocturnal leaf conductance.

2. Materials and methods

Two experiments were conducted: the first experiment (Exp. 1) aimed to clarify whether the decrease in the amount of submicron AS particles on the leaf surface of komatsuna plants over time after the end of exposure was affected by light. It has been reported that the uptake rate of solution from the leaf surface into the leaf interior was higher in opened stomata than in closed stomata (Eichert and Goldbach, 2008). The second experiment (Exp. 2) aimed to clarify the effects of submicron AS particles on the nocturnal leaf conductance of komatsuna plants. Experiment 2 comprised five sub-experiments (Exp. 2.1–2.5). Experiments 2.1–2.4 aimed to clarify the effects of submicron AS particles on the nocturnal leaf conductance (g_{\text{leaf}}) and cuticular conductance (g_{\text{cu}}) of komatsuna plants, and Exp. 2.5 aimed to determine the duration of the effects of submicron AS particles on the g_{\text{leaf}} of the plants.

2.1. Amount of SO4^{2−} deposited on leaf surfaces and its disappearance over time (Exp. 1)

2.1.1. Plant materials and growth conditions

Seeds of komatsuna plants (cv. Hakkei) were sown in seven cell-trays (16 cells per tray, each cell 62 mm long × 62 mm wide × 50 mm deep) filled with granular culture soil (N:P:K = 200:1223:150 mg l−1). In six out of seven trays, 12 cells per tray were used (i.e., 72 cells in total). In a remaining tray, eight cells were used for sawing, and the plants grown in this trey were used for the measurement of amount of cuticular wax and stomatal density of the leaf of the plants. After sawing, the trays were transferred into a naturally lit phytotron (approximately 6 m² with an air-conditioner system, Koito Industries Co., Ltd., Kanagawa, Japan) located at Fuchu Campus of Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan). In the phytotron, the air circulation was maintained at 60 m³ h⁻¹, air temperatures at 25.0 ± 1.0 °C/18.0 ± 1.0 °C (06:00–18:00/18:00–06:00), and relative air humidity at 70 ± 5%. Nine days after sowing (DAS), the plants were thinned to one plant per cell. At 21 DAS, komatsuna plants in four of the six trays were transferred into another phytotron and were exposed to submicron AS particles (AS treatment). The plants in the remaining two cell trays were not exposed to submicron AS particles (control). The phytotrons of AS and control treatments were controlled in the same environmental conditions described above. Air circulation and the air-conditioner system in the phytotron were stopped during the exposure to submicron AS particles, and were stopped for the same duration in the control. The plants were exposed to submicron AS particles twice; once at 03:00–04:00 and once at 04:30–05:30. It was required a 30 min break to recharge the silica gel. After exposure to submicron AS particles, the exposed plants were returned to the phytotron containing the control plants, and then the air circulation system in the phytotron was re-started. In the phytotron, half of the AS particle-exposed plants (i.e., plants in two of the four cell trays) were grown under light condition (approximately 300 µmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD) provided by blue- and red-LED lamps) (AS-light treatment). The other two cell trays of AS particle-exposed plants were grown under dark conditions (AS-dark treatment). The plants in the control were also grown under dark condition. During the experiment, the plants were carefully irrigated with tap water without splashing the leaf surface to avoid washing off the deposited particles. This experiment had three experimental replicates.

At 21 DAS, eight komatsuna plants grown in a tray were used for the measurements of the amount of cuticular wax and stomatal density of the third leaf of the plants (Table 1). Cuticular wax was extracted by

| Table 1 |
| Amount of cuticular wax on the leaf surface and stomatal densities on adaxial and abaxial leaf surfaces of komatsuna plants (cv. Hakkei) |
| Cuticular wax (µg cm⁻²) | Stomatal density (mm⁻²) |
| | adaxial | abaxial |
| 1.65 (0.18) | 166 (2) | 325 (21) |

Each value is the mean of four determinations, and the standard error is shown in parentheses.
immersing the leaf in chloroform (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 30 s, and then measured gravimetrically by the method of Sase et al. (2008). Stomatal density was measured by the stomatal impression method (Hirose et al., 1992).

2.1.2. Exposure to submicron AS particles

The komatsuna plants were exposed to submicron AS particles using the method described in our previous reports (Yamaguchi et al., 2014; Motai et al., 2017). The AS particles were generated from 0.1% (w/v) AS solution using an ultrasonic nebulizer (NE-U17, Omron Healthcare Co. Ltd., Tokyo, Japan), and were dried using a tape heater at 100°C and a diffusion dryer with granular silica gel. The mean size and number of AS particles was approximately 300 nm and 80,000 particles cm⁻², as measured using an aerosol measurement system based on a differential mobility analyzer and a particle counter (SMPS, TSI Incorporated, Shoreview, MN, USA). In the phytotron, komatsuna plants were exposed to submicron AS particles from approximately 50 cm above the plants with a slight downward wind provided by a fan (6.0 cm in length × 6.0 cm in width × 2.5 cm in height, 1.8 W, TUDCI2Z4, Nidec Servo Corp., Gunma, Japan) which was attached to the outlet of the aerosol exposure system. To uniformly expose the plants to submicron AS particles, two aerosol exposure systems were set on a rotating table with 1 turn per minute in the phytotron.

In Exp. 1, the concentration of SO₄²⁻ in PM₂.₅ (particulate matter with an aerodynamic diameter < 2.5 μm) during the exposure was not measured, but the average daily atmospheric concentration of SO₄²⁻ in PM₂.₅ inside the phytotron increased by the exposure was calculated using the method which was established in our previous study (Yamaguchi et al., 2014). In this study, PM₂.₅ inside the two phytotrons without any plants were collected using a filter holder with an impactor (flow rate at 20 L min⁻¹) in 2012. Exposure of AS particles and the measurement of PM₂.₅ inside the phytotrons were simultaneously started, and the exposure of the particles was conducted in one phytotron and not in another phytotron. After the exposure, exposure time and the sprayed volume of 0.1% AS solution were recorded. The measurement of PM₂.₅ inside the phytotrons was conducted for 24 h. The filters used for the collection of PM₂.₅ were washed with ultrapure water and SO₄²⁻ concentration in the washing solution was measured using an ion chromatography (883 Basic IC plus, Metrohm Japan Ltd., Tokyo, Japan). Then, the average daily atmospheric concentration of SO₄²⁻ in PM₂.₅ inside the phytotron was calculated. The difference in the average daily atmospheric concentration of SO₄²⁻ in PM₂.₅ between two phytotrons was assumed to be the actual increase in SO₄²⁻ in PM₂.₅ by the exposure. The procedure described above was repeated eight times and obtained a significant positive regression line between the average daily concentration of SO₄²⁻ in PM₂.₅ inside the phytotron and the volume of sprayed AS solution (R² = 0.818, p < 0.05, n = 8). Using this regression line, the average daily atmospheric concentration of SO₄²⁻ in PM₂.₅ inside the phytotron increased by the exposure was calculated as 16.5 μg m⁻³ in Exp. 1.

2.1.3. Measurement of amount of AS particles deposited on leaf surface

At 21–23 DAS, the amount of SO₄²⁻ deposited on the leaf surface of komatsuna plants was determined by the leaf washing method (Motai et al., 2017). Leaf washing was conducted at 0, 6, 12, 18, 30 and 48 h after the end of exposure to submicron AS particles (HAE), and leaf washing at 0 HAE was conducted at 06:00. At each measurement time, three plants per treatment were used. The third leaf of the plants was sampled and gently washed with 15 ml ultrapure water (18.2 MΩ cm at 25°C) for 1 min in a plastic bag. After washing, the area of the washed leaf was determined using image analysis software (LIA32, http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index.html). The washing water was filtered through a PTFE membrane (0.20 μm, DISMIC®-13HP, Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and then the concentration of SO₄²⁻ in the washing solution was determined by the ion chromatography. The amount of SO₄²⁻ deposited on the leaf surface was expressed per unit leaf area. The difference in the amounts of SO₄²⁻ on the leaf surface between the control and AS particle-exposed plants was regarded as the amount of AS particles deposited on the leaf surface by exposure to submicron AS particles.

Before conducting the experiment, we carried out a preliminary experiment to determine whether there was significant decrease in the amount of SO₄²⁻ deposited on an abiotic surface (PTFE membrane filter) over time after exposure. Thirty PTFE membrane filters were used in this experiment, with 15 of 30 filters assigned to the exposure to submicron AS particles. The washing procedure to determine the amount of SO₄²⁻ on the filter was carried out at 0, 6, 12, 30 and 48 HAE (n = 3 per treatment). As shown in Fig. 1, after the end of exposure to submicron AS particles, there was no significant decrease in the amount of SO₄²⁻ deposited on the filter over time.

2.2. Effects of AS particles on nocturnal leaf conductance and cuticular conductance (Exp. 2)

2.2.1. Plant materials and growth conditions

Seeds of komatsuna plants (cv. Hakkei) were sown in 2-L pots (diameter: 14 cm, height: 18 cm) filled with granular culture soil (12 pots in experiments 2.1–2.4, 18 pots in Exp. 2.5). After sowing, the plants in the pots were transferred into a naturally lit phytotron (approximately 6 m³ with an air-conditioner system) located at Fuchu Campus of Tokyo University of Agriculture and Technology. In the phytotron, the air circulation was maintained at 60 m³ h⁻¹, air temperatures at 25.0 ± 1.0°C/18.0 ± 1.0°C (06:00–18:00/18:00–06:00), and relative air humidity at 70 ± 5%. At 9 DAS, the plants were thinned to two plants per pot in Exp. 2.1–2.4 and one plant per pot in Exp. 2.5. In Exp. 2.1–2.4, komatsuna plants in six out of 12 pots (i.e., 12 plants) were transferred into another phytotron and then were exposed to submicron AS particles (AS treatment) as described in section 2.2.2. The plants in the other six pots were not exposed to submicron AS particles (control). The phytotrons of AS and control treatments were controlled in the same environmental conditions described above. Air circulation and the air-conditioner system in the phytotron were stopped during the exposure to submicron AS particles, and were stopped for the same duration in the phytotron in the control. The plants were exposed to submicron AS particles twice; once at 16:00–17:00 and once at 17:30–18:30. It was required a 30 min break to recharge the silica gel. After the end of exposure, the plants were...
returned to the phytotron containing the control plants. In Exp. 2.5, control or AS treatment had three phytotron replicates. Komatsuna plants were transferred into six phytotrons (three plants per phytotron). The plants in three of six phytotrons were exposed to submicron AS particles (AS treatment), and those in the other three phytotrons were not (control). The phytotrons of AS and control treatments were controlled in the same environmental conditions described above. The plants were exposed to submicron AS particles from 17:30–18:30. Air circulation and the air-conditioner system in the phytotron were stopped during the exposure to submicron AS particles, and were stopped for the same duration in the phytotron of control treatment. The plants were carefully irrigated with tap water without splashing the leaf surface to avoid washing off the deposited particles.

2.2.2. Exposure to AS particles

The AS particle exposure system and procedure are described in section 2.1.2. At 21 DAS, a transparent plastic chamber (approximately 1 m$^3$) was installed in the phytotron, and then komatsuna plants were exposed to submicron AS particles in this chamber. The chamber was only installed when the plants were exposed to the particles. The volume of the chamber during exposure in Exp. 2 was smaller than in Exp. 1, inducing the higher concentration of SO$_4^{2-}$ in PM$_{2.5}$. The average daily atmospheric concentration of SO$_4^{2-}$ in PM$_{2.5}$ increased by the exposure was calculated as 24.7 μg m$^{-3}$ using the method described in section 2.1.2.

2.2.3. Measurement of amount of AS particles on leaf surface

In Exp. 2.1–2.4, the amount of SO$_4^{2-}$ on the leaf surface of komatsuna plants was determined at 0 and 6 h after the end of exposure (HAE) to submicron AS particles by leaf washing as described in section 2.1.3. Three plants were randomly selected per treatment, and the third leaves were sampled for leaf washing.

2.2.4. Measurements of leaf conductance to water vapor

In Exp. 2.1–2.4, nocturnal leaf conductance to water vapor ($g_{\text{night}}$) and cuticular conductance ($g_{\text{cut}}$) of komatsuna plants were measured at 6 HAE (i.e., 00:30). Measurements of $g_{\text{night}}$ or $g_{\text{cut}}$ were conducted on the third leaves of randomly selected plants. The $g_{\text{night}}$ determination was performed with an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) at a leaf temperature of 18.0 ± 0.2 °C, atmospheric CO$_2$ concentration of 400 μmol mol$^{-1}$ and leaf-to-air vapor pressure deficit of 0.6–0.8 kPa.

The $g_{\text{cut}}$ (i.e., minimum epidermal conductance, $g_{\text{min}}$) was determined by the weight-loss method (Sack and Scoffoni, 2011). Immediately after detaching the third leaf, the cut petiole end was sealed with paraffin wax (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The leaves were placed in an incubator and dehydrated for about 1.5 h under dark conditions to induce turgor loss and stomatal closure. This duration was determined in a preliminary experiment. Then, the leaves were weighed with an electronic balance (CPA225D, Sartorius AG, Göttingen, Germany) and leaf area was determined using the image analysis software. This time was denoted as measurement time 0. After weighing, the leaves were placed back in the incubator. After 20 min, the leaves were weighed and the weight loss since the previous weighing was recorded. The leaves were re-weighed every 20 min, at least eight times. When the weight loss rate was stable, the leaf area was determined again. Air temperature and relative humidity were recorded every 15 s. The 20-min average air temperature (± standard deviation) during the measurement of $g_{\text{cut}}$ was 25.1 ± 0.1 °C, 24.4 ± 0.0 °C, 25.8 ± 0.0 °C and 25.8 ± 0.3 °C in Exp. 2.1, 2.2, 2.3 and 2.4, respectively. The 20-min average relative humidity (± standard deviation) during the measurement of $g_{\text{cut}}$ was 10.1 ± 0.6%, 15.2 ± 0.4%, 23.4 ± 0.2% and 23.2 ± 0.4% in Exp. 2.1, 2.2, 2.3 and 2.4, respectively. The $g_{\text{cut}}$ was calculated using the spreadsheet tool provided by Sack and Scoffoni (2011).

In Exp. 2.5, $g_{\text{night}}$ of the third leaf of komatsuna plants was measured with a leaf porometer (SC-1, Decagon Device Inc., Pullman, WA, USA). The leaf conductance was measured on the adaxial and abaxial leaf surfaces of the plants, and summed. The $g_{\text{night}}$ was measured at 1.5, 5 and 7 HAE during the nighttime in the phytotron (air temperature, 18 °C; RH, 70%). The time of day at 1.5, 5 and 7 HAE was 20:00, 23:30 and 1:30, respectively.

2.3. Statistical analyses

Statistical analyses were performed with the IBM® SPSS® Statistics 19 software package. Student’s t-test was used to identify significant differences between the control and AS treatments in Exp. 2.1–2.4, one-way ANOVA was used to test the significance of the difference in the experiment-pooled $g_{\text{cut}}$ or $g_{\text{night}}$ between the control and AS treatment, and each experiment was included as a random factor. In Exp. 2.5, three-way ANOVA was used to test the effects of submicron AS particles, leaf surface (adaxial or abaxial), and measurement time (1.5, 5 or 7 HAE) on $g_{\text{night}}$. When significant effects were observed, the significance of the differences among determinations was tested by Tukey’s HSD test.

3. Results

3.1. Decreasing trend of submicron ammonium sulfate particles deposited on leaf surfaces over time after exposure (Exp. 1)

Fig. 2 shows the trend in the amount of SO$_4^{2-}$ on the leaf surface of komatsuna plants over time, from 0 h after the end of exposure to submicron AS particles. Irrespective of the light conditions after exposure, the relative amount of removal of SO$_4^{2-}$ deposited on the leaf surface decreased over time. The relative amount of removal of SO$_4^{2-}$ deposited on the leaf surface was reduced to nearly 0% at 30 HAE and later. It seems that the rate of reduction in SO$_4^{2-}$ deposited on the leaf surface from 0 HAE to the first observation (6 HAE) in AS-light treatment was likely to be higher than that of AS-dark. The relative amount of SO$_4^{2-}$ deposited on the leaf surface of the plants was lower in the AS-light treatment than in the AS-dark treatment. At each measurement...
leaf conductance (submicron AS particles) signifi-
cantly abaxial surfaces of the third leaf of komatsuna plants. Exposure to
matsuna plants at 0 and 6 h after the end of exposure (0 and 6 HAE).

The amount of SO$_4^{2-}$ on the leaf surface of komatsuna plants (cv. Hakkei) at 0
and 6 hours after the end of exposure to submicron ammonium sulfate (AS)
particles (HAE) in Experiments 2.1–2.4

<table>
<thead>
<tr>
<th>HAE</th>
<th>Treatment</th>
<th>Amount of SO$_4^{2-}$ on leaf surface (nmol cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exp 2.1</td>
</tr>
<tr>
<td>0</td>
<td>Control</td>
<td>4.69 (0.24)</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>9.82 (1.23)</td>
</tr>
<tr>
<td></td>
<td>AS - Control</td>
<td>5.13 (1.23)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance</th>
<th>*</th>
<th>**</th>
<th>*</th>
<th>n.s.</th>
<th>***</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Control</td>
<td>4.30 (0.16)</td>
<td>2.13 (0.37)</td>
<td>4.27 (0.78)</td>
<td>2.63 (0.44)</td>
<td>3.35 (0.36)</td>
</tr>
<tr>
<td>AS</td>
<td>7.33 (2.34)</td>
<td>4.66 (1.31)</td>
<td>4.41 (0.89)</td>
<td>4.01 (0.63)</td>
<td>5.10 (0.73)</td>
</tr>
<tr>
<td>AS - Control</td>
<td>3.04 (0.16)</td>
<td>2.53 (0.37)</td>
<td>0.15 (0.78)</td>
<td>1.32 (0.44)</td>
<td>1.76 (0.63)</td>
</tr>
</tbody>
</table>

In each experiment, each value is the mean of three determinations. Average is
pooled value in Experiments 2.1–2.4 (n = 12). Standard error is shown in
parentheses. AS – Control indicates the amount of AS particles deposited by
the exposure on leaf surface. Student’s t-test and one-way ANOVA including
each experiment as a random factor were used in Experiments 2.1–2.4 and
in experiment-pooled average, respectively. Student’s t-test and ANOVA: *; p < 0.05, **; p < 0.01, ***; p < 0.001, n.s.; not significant.

Table 2 shows the amount of SO$_4^{2-}$ on the leaf surface of
komatsuna plants at 0 and 6 h after the end of exposure (0 and 6 HAE).
Immediately after exposure (0 HAE), significantly more SO$_4^{2-}$ was
deposited on the leaf surface of the plants in the AS treatment than in
the control treatment in Exp. 2.1–2.3 (Table 2). At 6 HAE, although
there was significantly more SO$_4^{2-}$ deposited on the leaf surface of AS-
exposed plants than on the leaf surface of control plants when
comparing experiment-pooled average values, there was no significant
difference in the amounts of SO$_4^{2-}$ between the control and AS treatments
in each individual experiment. The amount of SO$_4^{2-}$ deposited on
the leaf surface of the plants by the exposure (i.e., the difference in
the amount of SO$_4^{2-}$ on leaf surface of the plants between control and
AS treatments) was approximately 47% lower at 6 HAE than at 0 HAE.

Table 3 indicates the effects of submicron AS particles on nocturnal
leaf conductance (g$_{night}$) and cuticular conductance (g$_{cut}$) of the third leaf of komatsuna plants at 6 HAE. In Exp. 2.2, g$_{night}$ and g$_{cut}$ of the
plants were significantly higher in the AS treatment than in the control
treatment. Exposure to AS particles significantly increased the experi-
ment-pooled g$_{night}$, but did not significantly affect the experiment-
pooled g$_{cut}$.

Fig. 3 shows the effects of submicron AS particles on g$_{night}$ of adaxial and
abaxial surfaces of the third leaf of komatsuna plants. Exposure to
submicron AS particles significantly increased the g$_{night}$ of the plants.
The g$_{night}$ was significantly lower on the adaxial leaf surface than on the
abaxial leaf surface. There was a significant interactive effect between
AS particles and measurement time on g$_{night}$. When Tukey’s HSD test
was used to identify the interactive effect on g$_{night}$, the values were
pooled across the adaxial and abaxial leaf surfaces because there were
no significant interactive effects of leaf surface and AS treatment or time.
The g$_{night}$ of the AS-exposed plants at 1.5, 5 and 7 HAE were 82%,
55% and 32% higher than those of the control plants, respectively. At
1.5 and 5 HAE, the g$_{night}$ values were significantly higher for the AS-
exposed plants than for the control plants. At 7 HAE, however, there
was no significant difference in g$_{night}$ between the control and AS-exposed
plants.

Table 3 Effects of submicron ammonium sulfate (AS) particles on nocturnal leaf conduc-
tance (g$_{night}$) and cuticular conductance (g$_{cut}$) of the third leaf of komatsuna plants (cv. Hakkei) at 6 hours after the end of exposure to submicron AS particles in Experiments 2.1–2.4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exp. 2.1</th>
<th>Exp. 2.2</th>
<th>Exp. 2.3</th>
<th>Exp. 2.4</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>g$_{night}$ (nmol m$^{-2}$ s$^{-1}$)</td>
<td>AS</td>
<td>25.4 (5.10)</td>
<td>37.1 (5.81)</td>
<td>16.8 (5.13)</td>
<td>45.3 (5.13)</td>
</tr>
<tr>
<td>AS - Control</td>
<td>19.1 (4.77)</td>
<td>31.7 (5.83)</td>
<td>10.8 (4.77)</td>
<td>36.8 (5.83)</td>
<td></td>
</tr>
</tbody>
</table>

In each experiment, each value is the mean of three determinations. Average is
pooled value in Experiments 2.1–2.4 (n = 12). Standard error is shown in
parentheses. Student’s t-test was used in Experiments 2.1–2.4. One-way ANOVA
including each experiment as a random factor was used in experiment-pooled
average. Student’s t-test and ANOVA: *; p < 0.05, **; p < 0.01, n.s.; not signif-
icant.

Fig. 3. Effects of submicron ammonium sulfate (AS) particles on nocturnal leaf conductance (g$_{night}$) of adaxial and abaxial surfaces of the third leaf of komatsuna plants (cv. Hakkei) in Experiment 2.5. Each symbol represents the mean of three chamber replicates. Error bars indicate standard error. When Tukey’s HSD test was used to identify the differences between control and AS treatments, g$_{night}$ of adaxial and abaxial leaf surfaces were pooled because there were no significant interactive effects of leaf surface and AS treatment or time. Three-
way ANOVA: *; p < 0.05, **; p < 0.01, n.s.; not significant. The p-value is shown when 0.05 ≤ p < 0.10. Different letters indicate significant difference (p < 0.05. Tukey’s HSD test).

55% and 32% higher than those of the control plants, respectively. At
1.5 and 5 HAE, the g$_{night}$ values were significantly higher for the AS-
exposed plants than for the control plants. At 7 HAE, however, there
was no significant difference in g$_{night}$ between the control and AS-exposed
plants.

4. Discussion

After the end of exposure to submicron AS particles, the amount of AS particles deposited on the leaf surface of komatsuna plants decreased with time (Fig. 2 and Table 2), consistent with the results of our previous study (Motai et al., 2017). It has been reported that AS
particles deposited on the leaf surface of the plants absorb water vapor and deliquesce to form an AS solution, which is absorbed into the leaf interior via stomatal and cuticular pathways (Burkhardt et al., 2001; Eichert and Burkhardt, 2001; Eichert and Goldbach, 2008; Will et al., 2012). In our preliminary experiment, the amount of SO$_4^{2-}$ deposited on an abiotic surface (i.e., PTFE filter) did not significantly decrease with time (Fig. 1). Therefore, the reduction in the amount of SO$_4^{2-}$ deposited on the leaf surface could not be attributed to the volatilization of SO$_4^{2-}$, but instead reflected the uptake of SO$_4^{2-}$ into the leaf interior of komatsuna plants.

The uptake rate of SO$_4^{2-}$ deposited on the leaf surface of komatsuna plant from 0 to 6 HAE in AS-light treatment seemed to be higher than that in AS-dark treatments (Fig. 2). The contribution of stomata on foliar uptake is highly significant, and it was reported that the foliar uptake of aqueous solution was faster when stomata were open (Eichert et al., 2008; Fernández and Brown, 2013). Thus, the uptake rate of SO$_4^{2-}$ from 0 to 6 HAE in AS-light treatment was higher than in AS-dark treatment in the present study. However, there was no significant difference in the amount of SO$_4^{2-}$ deposited on the leaf surface of komatsuna plants between light and dark treatments through the entire measurement period for 48 h (Fig. 2). This result seemed to be contrary to the results reported by Eichert and Goldbach (2008). One of the possible reasons for this discrepancy is that nocturnal transpiration of komatsuna plants was high enough to induce the deliquescence of submicron AS particles deposited on the leaf surface. The relative air humidity near the leaf surface can be increased by transpired water vapor (Burkhardt et al., 1999). In addition, relatively high air humidity in the phytotron (70% RH) likely contributed to the deliquescence of submicron AS particles because the deliquescence point of AS is 79% RH (Cruz and Pandis, 2000). Another reason may be that stomata of the plants were not completely closed under dark conditions. Relatively high nocturnal stomatal conductance and incomplete stomatal closure during the nighttime have been reported for several plants (e.g., Musselman and Minnick, 2000; Snyder et al., 2003; Gruiske et al., 2004; Escalona et al., 2013). In the present study, the higher $g_{\text{night}}$ of komatsuna plants as compared with $g_{\text{cut}}$ was considered to result from incomplete stomatal closure (Table 3). As a result, the uptake rate of SO$_4^{2-}$ from the leaf surface to the leaf interior of the plants was occurred through stomatal pore even under the dark conditions. Thus, the uptake rate of SO$_4^{2-}$ from the leaf surface to the leaf interior of the komatsuna plants was not light dependent in the present study.

In Exp. 2.1–2.4, the experiment-pooled $g_{\text{night}}$ of komatsuna plants was significantly increased by exposure to AS particles (Table 3). This result is consistent with previous studies that suggested the increase in water loss from leaf by exposure to hygroscopic particles (Burkhardt et al., 2001; Pariyar et al., 2013; Burkhardt and Pariyar, 2016; Grantz et al., 2018). In the present study, $g_{\text{night}}$ includes both stomatal and cuticular conductance to water vapor. Water can be transported from the leaf interior to the atmosphere through the cuticle layer during the nighttime when the stomata are closed (e.g., Boyer et al., 1997). In the present study, therefore, it is possible that the AS particle-induced increase in $g_{\text{night}}$ of komatsuna plants was at least partly due to the increased $g_{\text{cut}}$. However, there was no significant effect of submicron AS particles on the experiment-pooled $g_{\text{cut}}$ (Table 3). Burkhardt and Pariyar (2014) reported that minimum epidermal conductance (i.e., $g_{\text{cut}}$) of Scots pine needles was significantly increased by spraying with salt solutions due to wax degradation. Because komatsuna leaves have much less cuticular wax than Scots pine needles (e.g., Kinnunen et al., 2001), we consider that wax degradation induced by the exposure to submicron AS particles did not strongly affect the $g_{\text{cut}}$ of komatsuna plants in the present study. The amount of cuticular wax and stomatal density of the leaf of komatsuna plants were determined as basic information of leaf characteristics (Table 1). This information will be useful when investigating the effects of hygroscopic materials such as AS particles on leaf water loss or comparing the effects of the particles among various plant species, because the particles may enhance leaf water loss through cuticle and/or stomata (Burkhardt et al., 2001; Grantz et al., 2018).

A significant interactive effect between AS-particles and time was detected for the $g_{\text{night}}$ of komatsuna plants, and the degree of increase in $g_{\text{night}}$ by exposure to submicron AS particles became smaller with time (Fig. 3). In the control, $g_{\text{night}}$ tended to increase with time, and was highest at 7 HAE; this trend seemed to reflect the circadian rhythm (Bucci et al., 2004; Easton and Richards, 2009; Resco de Dios et al., 2013). Conversely, $g_{\text{night}}$ of the plants in the AS treatment was highest at 1.5 HAE. The $g_{\text{night}}$ of the plants was significantly higher in the AS treatment than in the control at 1.5 and 5 HAE, but not at 7 HAE. The diminishing effect of submicron AS particles on the $g_{\text{night}}$ of the plants was related to the amount of SO$_4^{2-}$ on the leaf surface over time (Fig. 2 and Table 2). It is demonstrated that deliquescent salt solution connects leaf surface to the interior (Burkhardt and Pariyar, 2014; Burkhardt and Grantz, 2017). Burkhardt et al. (2001) reported that evaporation from inert filter was increased by application of NaNO$_3$ aerosol, indicating that the increase in water loss from leaf by hygroscopic salts is physicochemically induced. Thus, the reducing amount of SO$_4^{2-}$ on the leaf surface of the plants implies that the pathway of water transport which was established by deliquescent salt solution would decrease over time. Consequently, the degree of submicron AS particle-induced increase in $g_{\text{night}}$ of komatsuna plants would also become smaller over time. Although the hypothesis of HAS has not been completely proven yet, the results obtained in the present study seemed to support the hypothesis.

The values of $g_{\text{night}}$ shown in Table 3 (Exp. 2.1–2.4) were lower than those shown in Fig. 3 (Exp. 2.5). A possible reason for that is mainly attributed to the difference in the measurement methods of $g_{\text{night}}$ between Exp. 2.1–2.4 and Exp. 2.5. The open gas exchange system used in the Exp. 2.1–2.4 calculates the stomatal conductance using the steady-state water flux and leaf temperature. On the other hand, the leaf parameter used in the Exp. 2.5 predicts the stomatal conductance by an algorithm, and it likely induced relatively higher value of $g_{\text{night}}$, Thus, the difference of the measurement methods between Exp. 2.1–2.4 and Exp. 2.5 does not much influence the interpretation of the results obtained in the present study. Conversely, Motai et al. (2017) reported that the exposure to submicron AS particle-induced increase in $g_{\text{night}}$ because both Exp. 2.1–2.4 and Exp. 2.5 showed similar results (i.e., increase of $g_{\text{night}}$ by submicron AS particles).

In crop breeding, varieties with a low nocturnal transpiration rate are selected to improve water use efficiency (Cousel-Ledrut et al., 2016). Under future climate conditions, crop plants may face unexpected drought events due to climate change (IPCC, 2013). In addition, an elevated atmospheric CO$_2$ concentration might increase nocturnal stomatal conductance (Zeppel et al., 2012). Thus, increased nocturnal water loss from leaves due to hygroscopic aerosols such as AS particles may be an important issue for crop breeding in the near future. Furthermore, increased nocturnal water loss from leaves could lead to a disequilibrium of water potential between the soil and plants in the predawn period (Kavanagh et al., 2007), and adverse effects on plant water relations and productivity of crop plants (Pariyar et al., 2013; Motai et al., 2017). Conversely, Motai et al. (2017) reported that the exposure to submicron AS particles reduced the day-time stomatal conductance. Therefore, submicron AS particles may influence the water use and productivity of crop plants, especially in Asia where large amounts of anthropogenic aerosols are emitted.

5. Conclusions

After exposure to submicron AS particles, the amount of the AS particles deposited on the leaf surface of komatsuna plants reduced over time. The rate of removal of SO$_4^{2-}$ on the leaf surface of the plants under light condition was seemed to be higher than that under dark condition. However, the trend in this reduction did not differ significantly between the plants placed under light and dark conditions.
Exposing the plants to submicron AS particles significantly increased $g_{\text{night}}$, but did not significantly affect $g_{\text{day}}$. Thus, the submicron AS particle-induced increase in $g_{\text{night}}$ can be attributed to water loss from the leaf interior through stomatal pores. Although the degree of the submicron AS particle-induced increase in $g_{\text{night}}$ became smaller over time, the $g_{\text{night}}$ of AS particle-exposed plants was significantly higher than that of the control plants until 5 h after the end of exposure. Our results show that nocturnal water consumption of komatsuna plants is enhanced by submicron AS particles deposited on the leaf surface, and provide further information on the interaction between atmospheric aerosols and plants.

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References


+ epidermal + conductance + %28gmin%2C + a.k.a. + cuticular + conductance%29
(11 September 2017, date last accessed).


