

Release behavior of small sized daughter allergens from *Cryptomeria japonica* pollen grains during urban rainfall event

Q. Wang · S. Nakamura · S. Lu · G. Xiu ·
D. Nakajima · M. Suzuki · K. Sakamoto ·
M. Miwa

Received: 15 December 2010 / Accepted: 20 May 2011
© Springer Science+Business Media B.V. 2011

Abstract In Japan, *Cryptomeria japonica* pollen (with diameter $\sim 30\ \mu\text{m}$) is scattered during each spring season. Daughter allergenic particles, which are smaller in size than their parent pollen grain and are abundant in fine particles (the particle sizes $< 1.1\ \mu\text{m}$, $\text{PM}_{1.1}$), are released in the atmosphere. The daughter allergenic particles of pollen can be transported in the urban atmosphere for a long period of time after their release. In particular, the daily variation delays in the peaks of allergenic Cry j 1 concentrations compared with the peaks of airborne parent pollen counts were observed in high levels during 1 or 2 sunny days after rainfall. In addition, long range transportation of Asian dusts (ADS) from the East Asian continent was also

found during the pollen scattering seasons in Japan. Therefore, the interaction between pollen and air pollutants, including ADS, should be of concern. Thus, in this study, the morphological change of *Cryptomeria japonica* pollen and the elution behavior of its allergenic contents (Cry j 1) were investigated. Our results confirmed the existence of fine daughter allergen particles, which are clearly differ from the parent pollen grains in size. Fine allergenic particles in atmosphere were increased, while coarse allergenic particles were decreased on sunny days after rainfall. However, the correlation between the mass concentrations of fine particles and mass levels of Cry j 1 in coarse particles (the particle sizes $> 7.0\ \mu\text{m}$) was poor. The possible reason may be pollen burst at high humidity before rainfall. Additionally, Cry j 1 contents were emitted from the so-called Ubisch body, which contains allergenic Cry j 1 abundantly when pollen was in contact with rainfall. In particular, we found that 60% of allergenic Cry j 1 contents released in air polluted rainfall contained Ca^{2+} ion derived from road dust and ADS. Therefore, rainfall should be a main factor to induce transition of pollen allergenic contents to fine particles. In conclusion, allergenic particles which are small sized and translated into fine particles by rainfall can be inhaled into the lower respiratory tract and contribute to the hypersensitivity of asthma.

Q. Wang (✉) · S. Nakamura · G. Xiu ·
M. Suzuki · K. Sakamoto
Graduate School of Science and Engineering, Saitama
University, Saitama, Japan
e-mail: seiyo@mail.saitama-u.ac.jp

S. Lu (✉)
School of Environmental and Chemical Engineering,
Shanghai University, Shanghai, China
e-mail: senlinlv@shu.edu.cn

D. Nakajima
National Institute for Environmental Studies, Ibaraki,
Japan

M. Miwa
Center for Environmental Science in Saitama, Saitama,
Japan

Keywords *Cryptomeria Japonica* pollen · Pollen morphological changes · Allergens · Allergenic Cry j 1 · Suspended particulate matters (SPM)

1 Introduction

Japanese cedar pollinosis has been increasing in Japan and currently affects over 16% of the population (even up to 40% in the urban areas of Tokyo) (Nishihata et al. 1999). The prevalence is increasing in urban areas and it is currently about 28% (Bureau of social welfare and public health, Tokyo 2007). Since the number of hay fever patients has increased and the age of the patients with allergenic symptoms become younger, the tendency to the development of symptoms of asthmatic conditions has also increased (Maeda 2002). Particularly, the pollinosis has become a big social problem in recent years; therefore, an immediate remedy is desired. *Cryptomeria japonica* pollen, so-called Japanese cedar pollen, has two main allergens, Cry j 1 and Cry j 2, which are responsible for pollinosis. Allergenic Cry j 1 exists in the pollen wall (Ubisch body), while allergenic Cry j 2 presents as a starch granule on the pollen grain (Nakamura et al. 2004). According to our airborne sampling, Cry j 1 contents abundantly distribute in the size range ($PM_{1.1}$) and could be observed visually (Wang et al. 2008). It can be deduced that these allergenic contents are in the form of smaller particles and can be released from coarse particles of original *Cryptomeria japonica* pollen grains (about 30 μm) even to fine allergen particles below 1.1 μm ($PM_{1.1}$). Therefore, these can be called daughter allergen particles of *Cryptomeria japonica* pollen grains. Cry j 1 suspended fine particles were observed during sunny days after rainfall. In the Saitama urban residential area, pollen grains and allergenic daughter particles on the asphalt may also be resuspended by heavy traffic volumes. Morphological of pollen grains can be changed by air pollutants in atmosphere, such as suspended particulate matters (SPM), rain water (its pH is different before and after rain), the amount of allergenic contents from pollen grains through broken pollen walls, and acidification of the allergen can be changed (Motta et al. 2006; Behrendt et al. 1997; Rogerieux et al. 2007). In addition, the long range transportation phenomena of Asian dust particles (ADS) transported by the westerlies from the East Asian continent was reported during pollen scattering season in the recent years. When the ADS was transported to Japan, ionic concentrations in rainfall were highly increased. Especially, Ca^{2+} was increased because of soil particles emitted from desert areas of the northern China where are the emission sources of

alkaline ADS (Suzuki and Tsunogai 1988). Ca^{2+} has been reported to effect on the pollen germination and extension of the pollen tubes (Holdway-Clarke et al. 2003). Therefore, the interaction between pollen and different air pollutants should be of a concern. Actually, pollinosis tended to aggravate when the pollen coexisted with soil particles, such as Kanto loam local dust around Tokyo, Japan (Maejima et al. 2001), and the ADS were inhaled in the mean time (Ichinose et al. 2009). Although the information of airborne *Cryptomeria japonica* pollen was reported by other researchers (Goto 2010; Okuda et al. 2006), no research focused on the morphological change of *Cryptomeria japonica* pollen grains and the release mechanism of their allergenic contents by various inorganic ionic components during rainfall until now. In this study, we investigated the morphological variation of *Cryptomeria japonica* pollen grains, analyzed the elution behavior of their allergenic contents, and focused on the characterization of small sized daughter particles derived from their parent pollen grains when they come into contact with the simulated polluted rainfalls of the urban atmosphere containing various inorganic ionic components.

2 Materials and methods

2.1 Sampling for atmospheric particles in urban area

One Andersen high-volume air sampler (AH-600, Shibata Scientific Technology Co., Ltd.) was used to collect atmospheric particles during the pollen scattering seasons (from February to March) of 2007, 2008 and 2009. *Cryptomeria japonica* pollens, their allergenic daughter particles and SPM were collected on quartz fiber filters before and after rainfall.

This sampler can collect size-resolved ambient particles (the particle sizes <1.1, 1.1–2.0, 2.0–3.3, 3.3–7.0 and >7.0 μm). Because pollen allergens may exist in fine particles (the particle sizes <1.1 μm , $PM_{1.1}$) and coarse particles (the particle sizes > 7.0 μm) abundantly, we then measured allergen concentrations in both ranges of $PM_{1.1}$ and particle with diameter larger than 7.0 μm . Air flow of the sampler is 566 L/min for each 71 h during the sampling periods of 2007 and for each 47 h during the sampling periods of 2008 and 2009.

Because forecast of *Cryptomeria japonica* pollen counts in 2007 was very low, pollen was collected for 71 h. For these sampling filters, we measured allergenic Cry j 1 and inorganic ionic concentrations in these collected particles.

In order to calculate the amount of pollen and observe their morphology in the atmosphere, airborne pollens were also collected on a glass slide coated with white petrolatum and carbon double-sided tape by the Durham sampler (DK-1SA, Nishiseiki Co., Ltd.), which is standard method in Japan. The meteorological data (precipitation) was provided by Saitama Institute of Public Health which located near our sampling site (Fig. 1).

2.2 Visualizing methods for confirming fine daughter allergen particles

In order to confirm fine daughter allergen particles, fine particles ($PM_{1.1}$) were investigated by using a scanning electron microscopy (SEM) under ultra low-pressure (vacuum) and an immunofluorescence (green) technique with a fluorescence microscope. Cry j 1 can be specifically identified by using anti-Cry j 1 mouse monoclonal antibody (Cry j 1 MAb), which has been developed and reported by Wang et al. (2008). In this method, the primary antibodies of Cry j 1 MAb and FITC (Fluorescein isothiocyanate) conjugated goat anti-mouse IgG (Jackson Immuno research Laboratories, Inc.) were used as a fluorescence protein to label allergenic Cry j 1 contents specifically.

2.3 Exposure experimental design and chemical analyses of pollen and SPM

The exposure experiments consisted of two exposure studies, one experiment was fresh pollen grain samples above carbon double-sided tape (provided by Japan Automobile Research Institute) were exposed to heavy traffic and polluted urban atmosphere on the roadside for 9 h. The purpose was to find out any morphological changes on pollen surface or vitality. After the exposure, pollen grains were collected and preserved for surface observation. Another exposure equipment was carried out in the laboratory for analyzing adsorption of gaseous pollutants (Gas monitors for NO_2 (Model-265p), SO_2 (FLAT1000) and NH_3 (Model 17)) on the pollen grains in an exposure experimental chamber.

2.4 Observation of morphological change of *Cryptomeria japonica* pollen using an environmental scanning electron microscope

Samples would be investigated with a SEM under ultra low pressure (vacuum). Because the regular vacuum pressure (10^{-8} – 10^{-3} Pa) in the traditional SEM may cause pollen to burst, an environmental-SEM (ESEM, Model Quanta 200), FEI Company Co., Ltd.) was employed to observe morphological change of pollen samples under low vacuum (10–2,000 Pa) in this study. Due to the difference of pressures in internal and external pollen is so little, the possibility of pressure-induced pollen burst was very slim. Working parameters, including humidity, temperature, pressure, and injection of solution or gas components, can be changed in the sample chamber of the ESEM. With the help of this new technology, we can investigate the mechanism of pollen burst only caused by high humidity. After the pollen samples were put in the sample chamber of the ESEM, the morphological change of the pollen grains was observed. The working conditions of this studies were set at 600–850 Pa and the humidity varied from 20 to >100% in the chamber. Microscopy of the pollen grains was recorded at every 5 s, and then these pictures were cascaded to make a film. In addition, the morphological change of pollen was investigated before and after the pollen grains were treated with 1,500 mI $Ca(NO_3)_2$. Disseminated pollen grains on carbon double-sided tape were also treated with 1,500 mI $Ca(NO_3)_2$ solution, which was fed into the sample room. After the pollen was contacted with solution, the humidity was reduced and the sample was dried in the sample room. Finally, the pollen was ready for observation under the ESEM.

2.5 Elution of pollen allergen by contacted with simulated rains

In order to confirm the transition of allergenic Cry j 1 contents to fine particle sizes after they were contacted with rainfall, 500 mg pollen samples were mixed with KNO_3 , NH_4NO_3 , $Ca(NO_3)_2$, NH_4Cl , $(NH_4)_2SO_4$, $(NH_4)_2C_2O_4$, ultrapure water, and extraction solution, respectively. The pH value of these simulated rains was 4–5 (average pH of rainfall in Japan). Moreover, when ADS transported to Japan,

pH was reported to be increased (above 7.0) with Ca^{2+} . Therefore, we selected $\text{Ca}(\text{NO}_3)_2$ as scheming ADS. The extraction solution was usually prepared for the effective elution of allergenic Cry j 1 content from pollen grains (Takahashi et al. 2001). The ionic strengths of these inorganic salts were 80, 150, 500, and 1,500 mI, respectively. After the *Cryptomeria japonica* pollen solutions that were made from eluted allergen contents was filtrated by using a cellulose acetate membrane filter (pore size, 1.2 μm), the pollen solution was centrifuged for 10 min at 15,000 rpm. The amount of 1 mL of the supernatant was picked and exchanged to the buffer solution (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20) by a centrifugal filter unit (amiconultra-4, Millipore). These samples were stored below -40°C until the measuring experiment.

2.6 Measurement methods of allergenic Cry j 1 concentrations

The ELISA (enzyme-linked immunosorbent assay) method and the surface plasmon resonance method (SPR) (Biacore J system, GE Healthcare Co. Ltd) were used for the biochemical analyses, mainly based on the immunology to detect the presence of the antibody or its antigen in a sample. The positive correlation between ELISA and SPR methods has been reported (Cho and Park 2006).

Atmospheric Cry j 1 collected by an AH-600 air sampler was measured using the ELISA method. Allergenic Cry j 1 contents were extracted using the following method. Briefly, the quartz fiber filters were saturated in 3 mL of Cry j 1 extraction solution (150 mM of NaCl, 0.125 M of NH_4CO_3 , 3 mM of EDTA, 0.005 wt% of Tween 20, and 10 mM of HEPES buffer solution) for overnight at 4°C and then shook at 192 rpm for 1 h. These samples were centrifuged for 30 min at 6,000 rpm, and supernatant of these solutions were picked up. Finally, allergenic Cry j 1 concentrations of these supernatants were measured by the routine ELISA method and protocol (Sawatani et al. 1994).

Cry j 1 concentrations eluted from the pollen samples in contact with simulated rain were measured with a Biacore J system. This measuring method was adopted from the method of Takahashi et al. (2001) with the improved procedure simply as followed. Allergenic Cry j 1 concentrations in

measured samples were investigated based on Cry j 1 MAb immobilized on a CM5 sensor chip (GE Healthcare Co.) by using the SPR method with the Biacore J system. About 700 μg of Cry j 1, MAb was immobilized on the chip. The regeneration and dissociation of antigen–antibody complex reaction on the CM5 sensor chip were performed by 70 μL of pH 2.0 glycine. Finally, the results were converted into allergenic Cry j 1 concentrations eluted from 1.00 mg pollen grains ($\mu\text{g}/\text{mg}$) in mass.

2.7 Measurement method of pollen counts

In order to count the pollen grains in the atmosphere, airborne pollens were collected on a glass slide coated with white petrolatum and carbon double-sided tape by the Durham samplers (DK-1SA, Nishiseiki Co., Ltd.), which is standard pollen collecting method in Japan. The principal of this instrument to collect airborne pollens is based on gravitational sedimentation. In order to compare pollen counts and Cry j 1 concentration collected by the AH-600 air sampler, airborne pollen was collected for each 23 h.

The pollen counts (pollens/ $\text{cm}^2/23\text{ h}$) in the slide glass covered with cover glass, 18 mm $\times$ 18 mm, were carried out using the calculation of the pollen dyed by Phöbus Blackly distain solution which made from 0.6 mg of methyl violet 2B, 30 mL of phenol, 180 mL of glycerin, and 90 mL of ultrapure water and by using an optical microscope (Shimadzu Co., Ltd.) under 400 magnifications (Fig. 1).

2.8 Measurement method of various ionic concentrations

Aqueous ions, including Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} , F^- , Cl^- , NO_3^- , SO_4^{2-} , extracted by ultrasonic extraction with 40 mL of ultrapure water for 20 min from the quartz fiber filters hollowed out 30 pieces (diameter of the pieces = 8 mm) were measured by an ion chromatograph (DX-100, Dionex, CA, USA). Ion concentrations were converted into ionic concentration ($\mu\text{g}/\text{m}^3$) in the urban atmosphere using following Eq. (1).

$$C(\mu\text{g}/\text{m}^3) = (C_s - C_b) \times \frac{F}{F_a} \times V_e \div V \quad (1)$$

In this equation, C_s was sample concentration ($\mu\text{g}/\text{mL}$), C_b was filter blank concentration, F was total



Fig. 1 Sketch map of sampling site

filter area (mm^2), F_a was area of filter which was measured (mm^2), V_e was quantity of ultrapure water which used extraction (mL), and V was collected total volume (m^3).

3 Results and discussions

3.1 Airborne behavior of pollen counts and size-segregated allergen particles before and after rainfall

Figure 2 indicates time variations of allergenic Cry j 1 concentrations and pollen counts during March 10th–23rd, 2008 in Saitama, Japan as example of tendency of scattering allergen and pollen. The allergenic Cry j 1 concentrations in the atmosphere were mainly found in $\text{PM}_{1.1}$. In particular, Cry j 1 concentrations in $\text{PM}_{1.1}$ were increased on sunny days after heavy rain (26 mm rainfall during March 19th–20th). Other rainy days included March 10th (8 mm rainfall) and March 14th (5 mm rainfall). Since rainfall on March 10th occurred in the morning, which was before pollen scattering time, and it happened from 16:00 on March 14th, which was after pollen scattering time, the effect of decreases in pollen counts and Cry j 1 concentrations were few. *Cryptomeria japonica* pollen scattered from *Cryptomeria japonica* tree was generally scattered from day time, about 6:00–18:00 at February to April in Japan. It was called washout that SPM can be removed by rainfall. In addition, SPM was extremely decreased during rainfall event. Cry j 1 concentration ratios

between $\text{PM}_{1.1}$ and coarse particles (the particle sizes $> 7.0 \mu\text{m}$) before and after rainfall in 2007–2009 are shown in Fig. 3. Cry j 1 concentration ratios increased after rainfall compared with that before rainfall. The correlation of Cry j 1 contents with $\text{PM}_{1.1}$ and with coarse particles (the particles sizes $> 7.0 \mu\text{m}$) after rainfall was quite poor ($R = 0.31$, $n = 20$), whereas a good correlation of Cry j 1 concentrations with $\text{PM}_{1.1}$ and coarse particles (the particle sizes $> 7.0 \mu\text{m}$) before rainfall was exist ($R = 0.90$, $n = 20$). We noticed that average pollen counts before rainfall were 167.6 ± 137.3 (counts/ $\text{cm}^2/23 \text{ h}$), while the average pollen counts after rainfall were 125.0 ± 84.4 (counts/ $\text{cm}^2/23 \text{ h}$). Therefore, Cry j 1 concentrations in $\text{PM}_{1.1}$ after rainfall could possibly be contributed by something rather than pollen counts suspended in the atmosphere. Bartkova-Scevkova (2003) reported that the pollen counts such as *Betula*, *Poaceae*, and *Ambrosia artemisiifolia* were also reduced after rainfall days. Schappi et al. (1999) also demonstrated that fine birch allergen particles increased at rainfall. In our field observation, the results of *Cryptomeria japonica* pollen and allergenic contents were of similar phenomena in urban atmosphere of Japan. Schappi et al. (1997) examined pollen which had been in contact with rainfall and found the growth of pollen tubes and the release of starch granules containing allergenic contents. Thus, it could be seen that allergenic particles among fine particles were from resuspension on sunny days after rainfall. Because allergen particles release from pollen were different with pollen morphology, these allergen particles were impossible to be count as pollen under optical microscope. Therefore, high allergenic particle concentrations after rainfall may be caused by two emission reasons. One reason was the increment of pollen grains emitted directly from *Cryptomeria japonica* trees, and another might be the release of fine allergenic particles emitted indirectly from the pollen grains with their morphological change during the transportation. In addition, fine allergenic particles were impressionable by airflow such as auto mobile traffic. Therefore, we could conclude that one of the factors which caused mass level of fine allergenic particles increase on sunny days after rainfall was fine allergenic particles from pollen grains that were released by rainfall and resuspended in the urban atmosphere.

3.2 Visualization of fine daughter allergenic particles in PM_{1.1}

In Fig. 4a and b, Ubisch bodies contained Cry j 1 were observed by SEM, which was released from its original pollen grain. In addition, green fluorescence luminescent spots on the quartz fiber filters could be found under the immunofluorescence microscopy (Fig. 4c). Moreover, ruptured pollen of content inside pollen that contains allergen (Fig. 4d) and exfoliative Ubisch bodies on pollen surface (Fig. 4e) was observed.

Therefore, Cry j 1 contents distributed below 1.1 μm in size and they clearly differed from pollen grains with size about 30 μm . From these results, we can confirm the existence of fine daughter allergen particles of exfoliated Ubisch bodies, which suspended in the atmosphere, can be inhaled into the lower human respiratory system tract.

3.3 Morphological observation for pollen grains

In order to investigate the effects of air pollutants on pollen grains, we have conducted an exposure experiment of pollen to air pollutants on roadside. After 9 h exposure, pollen was analyzed by SEM. The morphological feature of the pollen is shown in (Fig. 4). We found that agglomeration of particles attached to pollen surfaces did not reveal any changes in pollen structure or vitality in spite of the exposure for a period of time up to 9 h. The particles attached to pollen surfaces are crystalloid (Fig. 5a and b). Therefore, we inferred it might be secondary particles such as ammonium salts formed by acidic particles, gases, and other alkaline substances.

Fig. 2 Time variation of Cry j 1 concentrations and pollen counts (March 10th ~ 23rd, 2008) in Saitama, Japan

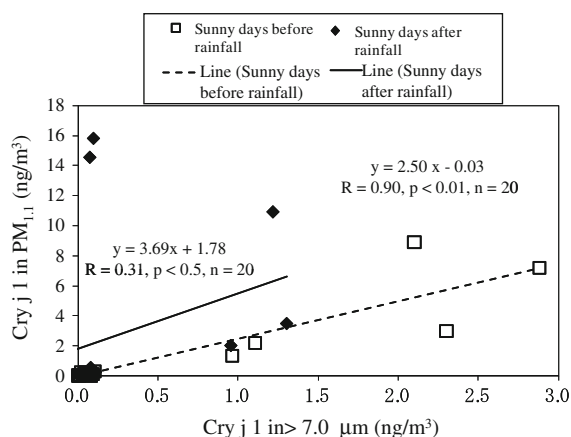
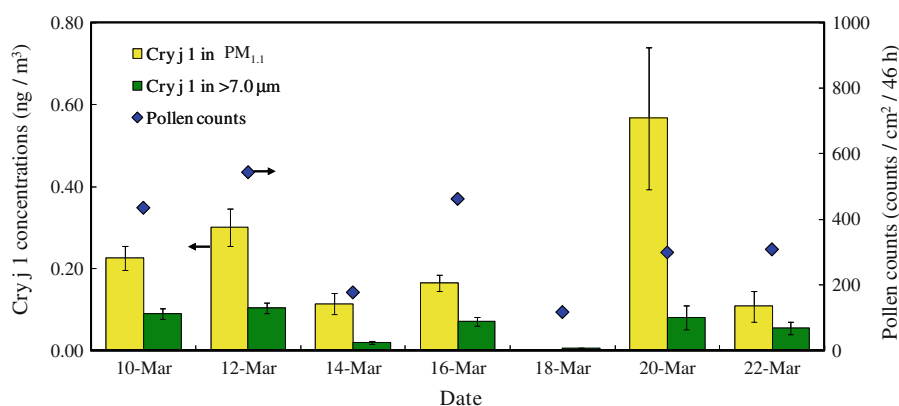


Fig. 3 Comparing Cry j 1 concentration ratios between fine particles (PM_{1.1}) and coarse particles (>7.0 μm) before and after rainfall based on our field investigations

3.4 Influence of exposure gaseous and particulate pollutants

Figure 6 indicated inorganic ionic concentrations in SPM of our sampling site. Inorganic ions and secondary formation (NH_4^+ , NO_3^- , and SO_4^{2-}) were at high concentrations. Thus, various pollutants and automobile emission particles were possible to agglomerate and deposit on pollen surface. Okuyama et al. (2007) investigated agglomeration of inorganic particles on pollen. From our laboratory experimental results, it can be seen that gaseous pollutants (NO_x , SO_2 , and NH_3) (shown in Fig. 7) were adsorbed to the fresh pollen samples. Different crustal substances adsorbed to SPM could mediate agglomeration of particles were deposited on the pollen surfaces followed by modification of pollen and, then, under appropriate conditions (e.g., humidity). As the results, it might induce

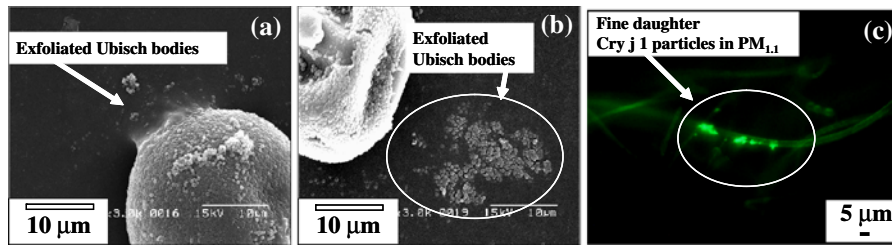


Fig. 4 Confirmation of fine daughter allergen particles (a) and (b) by using of SEM and an immunofluorescence technique (c)

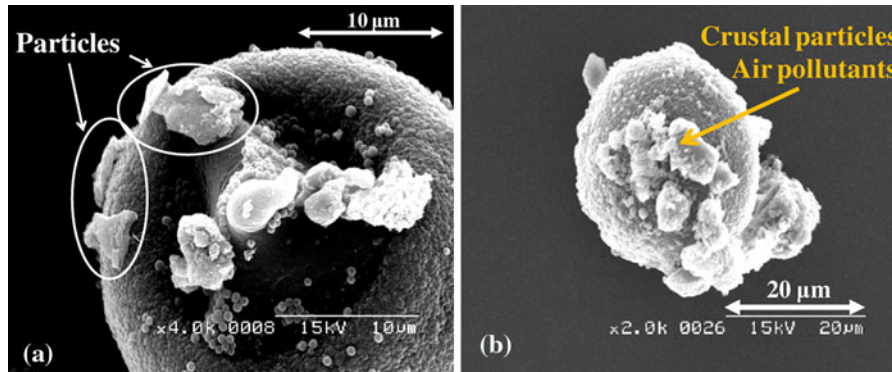


Fig. 5 Scanning electron micrograph (SEM) of ambient pollen surface which particulates are agglomerated onto the surface exposed to air pollutants and crustal particles in Urawa urban area, Saitama city, Japan

circumscribed local allergen release. Therefore, ambient pollens are not only of bioaerosols but also of pollutant carriers in high polluted atmosphere. The electron microscopy experimental results showed that SPM soluble solution can induce the release of allergen proteins from pollen grains and give rise to the formation of fine allergen particles (the particle sizes $< 1.1 \mu\text{m}$) (Wang et al. 2008).

3.5 Morphological change of pollen grain with high humidity

The burst phenomena of *Cryptomeria japonica* pollen could be observed under ESEM. When humidity in the chamber increased, the pollen grains began to swell. About 4 min later, the humidity in the chamber reached 100% the pollen burst and released the inner cells (Fig. 8). After sizes of normal pollen grain (Fig. 8a) and adjacent burst (Fig. 8b) were compared, normal pollen size was measured as $26.37 \mu\text{m}$, whereas pollen size at adjacent burst enlarged to $32.56 \mu\text{m}$. Because the allergenic contents contained in starch granules, pollen burst by high humidity before rainfall also contributed to an increase in fine allergenic particles.

3.6 Morphological changes of pollen grains when contacted with a simulated rain contained $\text{Ca}(\text{NO}_3)_2$

From the morphological observation shown in Fig. 9, Ubisch bodies with the size of approximate hundreds of nanometers can be found on the surface of pollen. After the pollen grains contacted with Ca^{2+} rich solution, Ubisch bodies (Fig. 9d) were eluted and the surface of pollen grain became smooth. Allergic Cry j 1 contents existed in Ubisch bodies of *Cryptomeria japonica* pollen, and their size can be down-sized to fine daughter allergenic particles. Therefore, rainfall is regarded to link with the release of fine allergenic particles.

3.7 Investigation of eluted concentration of allergenic Cry j 1 from *Cryptomeria japonica* pollen

Allergenic Cry j 1 concentrations from *Cryptomeria japonica* pollen which have been eluted with various simulated rains are indicated in Fig. 10. The broken lines show the ballpark allergenic Cry j 1 contents,

Fig. 6 Average mass level of inorganic ions in ambient particles before rainfall

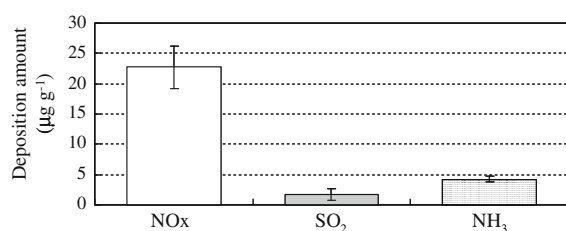
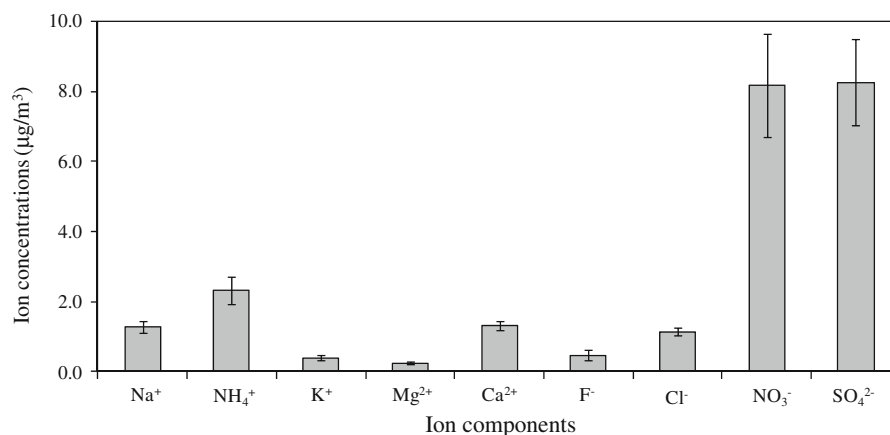


Fig. 7 Amount of deposition of NO_x, SO₂, NH₃ exposed to *Cryptomeria japonica* pollen ($n = 3$). The bars show the standard deviation

which were the analysis result of extraction solution. Comparing the cation (Fig. 10a) and anions (Fig. 10b) effects, we noticed the Cry j 1 contents were most effectively eluted in simulated rain water

containing Ca²⁺ ion. About 60% of the allergenic Cry j 1 concentrations were eluted from pollen by Ca²⁺ ion-containing rain compared with the extraction solution. When extraction solution containing anion was used to investigate allergenic Cry j 1 release, simulated rain containing (NH₄)₂SO₄ was more effective than other anions for the elution. However, (NH₄)₂SO₄ was not affected by Ca²⁺ ions. This study revealed that allergenic contents from *Cryptomeria japonica* pollen were more eluted when in contact with rain water containing air polluted ions. Moreover, rainfall contained mineral ion, Ca²⁺, derived from Asian dust and crustal particles has a greater elution effect. However, because eluted allergenic

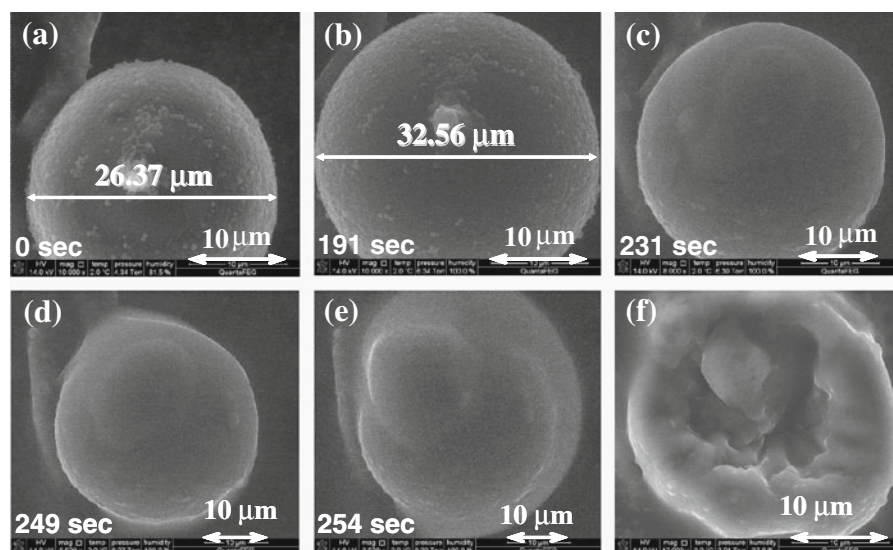
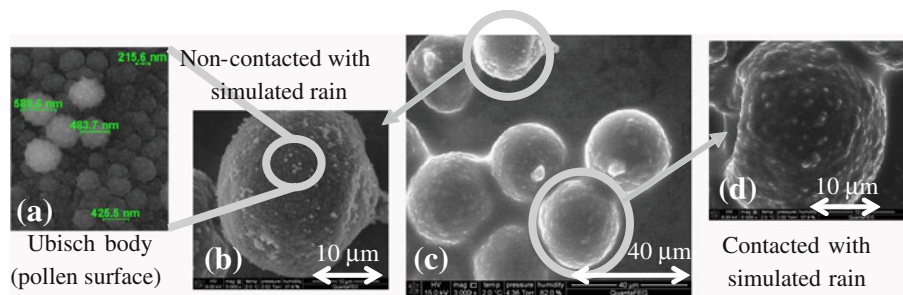


Fig. 8 Morphological changes of pollen under **a** low humidity; **b** high humidity; **c** more swelled; **d** pollen burst; **e** Inner cells diffused; and **f** after burst

Fig. 9 Morphological change of pollen grain by a SEM, **a** Ubisch bodies approximately hundreds of nanometers; **b** original pollen grains (noncontacted with solution); and **c** and **d** pollen grains contacted with 1,500 mI $\text{Ca}(\text{NO}_3)_2$ solution, respectively



concentrations were decreased when the simulated rains had few ions and high ionic strength, dust particles transported long distance from Asian continent to Japan contains more ionic composition; these kinds of particles will be used to investigate allergen daughter particle release ability from pollen grains after they contact with the particles solution in our further study.

4 Discussion and conclusions

We investigated the airborne behavior of *Cryptomeria japonica* pollen and its small sized allergenic particles, the so-called daughter allergen. Cry j 1 concentration ratios between fine and coarse particles were increased after rainfall compared with before rainfall. Nevertheless, coarse allergenic particles and pollen counts were decreased and the relationship between Cry j 1 concentrations and coarse particles ($>7.0 \mu\text{m}$) after rainfall were poor. Therefore, fine allergen particles after rainfall were increased by pollens suspended before rainfall and were taken up by raindrops and eluted as fine allergenic particles. Original particle size of *Cryptomeria japonica* pollen grains was about $30 \mu\text{m}$. However, the pollen allergenic particles were not only determined in coarse particles of the original *Cryptomeria Japonica* pollen grains but also in the small size particles in the atmosphere (Rantio-Letimäki et al. 2002). However, the release process of pollen allergen was not reported. In this paper, we firstly studied small sized phenomena of *Cryptomeria japonica* pollen allergens and daughter allergenic particles released from its original pollen by rainfall. Our field sampling studies suggested that rainfall was one of the forces for this release. To verify our hypothesis, we have conducted elution experiments with simulated polluted rain. Since pollen grains are easily to get burst under high vacuum condition of

traditional SEM. The ESEM which has ultra low-vacuum condition was employed to investigate the pollen burst phenomena with the change of humidity. We confirmed the burst of *Cryptomeria japonica* pollen under the high humidity. In addition, morphological changes and the behavior of eluted allergenic concentrations from the original pollen in simulated rain solutions which contain different cations and anions were also investigated. Our results demonstrated that the daughter allergenic particles may be released as fine particles from their parent pollen during sunny days after Ca^{2+} rich rainfall, in spite of the decrease in pollen counts. Additionally, we discovered the released allergenic particles concentrations from pollen were increased (60%) when the sample was in contact with simulated Ca^{2+} containing alkaline rain water, which is likely produced from road dust and Asian dust from the East Asian continent.

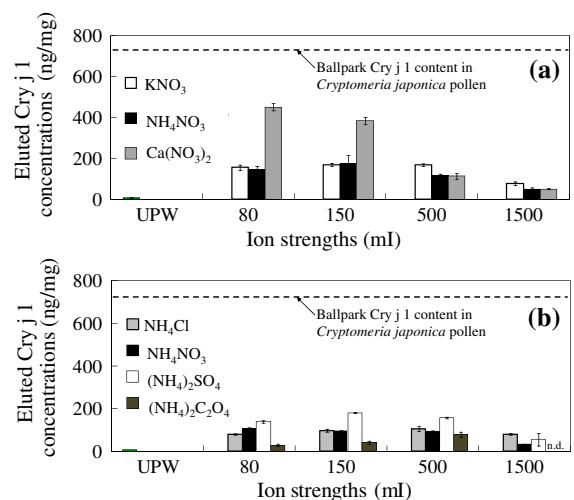


Fig. 10 Tendency of eluted Cry j 1 concentrations in various ion strengths and salt components, when cations (a) and anions (b) contents and ion strengths were changed. Broken lines are ballpark Cry j 1 content analyzed by solution that elute from pollen grain effectively. UPW stands for ultrapure water

Wang et al. (2009), Kurihara et al. (2007), and Okuyama et al. (2007) also reported that the deposition on the surface of pollen grains in urban area was adhered with other airborne particles, such as crustal particles and/or secondary formed particles. Therefore, complex airborne particles, such as pollen containing with other particles, will induce regional high ionic strengths on their surfaces when they are in contact with rain drops and moisturized. Especially, pollens which are adhered with secondary formed particles encompassing deliquescence, such as NH_4NO_3 , are of possible serious effect. Sagehashi et al. (2005) reported that Cry j 1 was easy to adhere on hydrophobic surface. Namork et al. (2006) also reported that pollen allergens were adhered carbon aggregates in atmosphere from vehicle exhaust. Therefore, generation of carbon aggregates by heavy traffic in urban areas will result in resuspension of eluted Cry j 1. These results indicate that allergenic particles that are down-sized to fine particles by rainfall may contain abundant aqueous inorganic ions. Therefore, we conclude that air polluted rainfall will induce the small sized *Cryptomeria japonica* pollen allergens. It was worried that these pollen allergenic contents were dried during sunny days; moreover, the allergenic particles can be resuspended and turn into fine particles by wind or heavy traffic in the urban residential atmosphere. Additionally, these fine allergenic particles will be inhaled into the lower respiratory tract and contribute to the hypersensitivity of asthma.

Acknowledgments Some works of this study are supported by the Special Funds for Basic Research (B) (No. 17310031, FY2005–FY2007) and Innovative Area Research (No. 20120015, FY2008–FY2013) of Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the FY2007–FY2008 Research project of Innovative Research Organization, Saitama University, Japan, Shanghai Pujiang Talent program, Innovation Program of Shanghai Municipal Education Commission (Grant No. 11ZZ80).

References

- Bartkova-Scavkova, J. (2003). The influence of temperature, relative humidity and rainfall on the occurrence of pollen allergens (*Betula*, *Poaceae*, *Ambrosia artemisiifolia*) in the atmosphere of Bratislava (Slovakia). *International Journal of Biometeorology*, 48, 1–5.
- Behrendt, H., Becker, W. M., Fritzsche, C., Sliwa-Tomczok, W., Tomczok, J., Friedrichs, K. H., et al. (1997). Air pollution and allergy: Experimental studies on modulation of allergen release from pollen by air pollutants. *International Archives of Allergy and Immunology*, 113, 69–74.
- Bureau of social welfare and public health, Tokyo metropolitan government. (2007). *The report of the actual condition for pollinosis sufferers* (pp. 22–25) (in Japanese).
- Cho, H., & Park, N. (2006). Serodiagnostic comparison between two methods, ELISA and surface Plasmon resonance for the detection of antibodies of classical swine fever. *Japanese Society of Veterinary Science*, 68(12), 1327–1329.
- Goto, M. (2010). A survey of airborne Japanese cedar pollen in the kansai region—10-year survey results from Hyogo prefecture. *Seikatsu Eisei*, 54(1), 3–12. (in Japanese).
- Holdway-Clarke, T. L., Weddle, N. M., Kim, S., Robi, A., Parris, C., Kunkel, J. G., et al. (2003). Effect of extracellular calcium, pH and borate on growth oscillations in *Lilium formosanum* pollen tubes. *Journal of Experimental Botany*, 54, 65–72.
- Ichinose, T., Hiyoshi, K., Yoshida, S., Takano, H., Inoue, K., Nishikawa, M., et al. (2009). Asian sand dust aggravates allergic rhinitis in guinea pigs induced by Japanese cedar pollen. *Inhalation Toxicology*, 21(12), 985–993.
- Kurihara, K., Wang, Q., Kiryu, H., Sakamoto, K., Miwa, M., & Uchiyama, I. (2007). Airborne behavior of suspended particulate matters containing Japanese cedar pollen allergens in urban, roadside and mountain areas of Saitama, Japan. *Journal of Japan Society for Atmospheric Environment*, 42, 362–368. (in Japanese).
- Maeda, Y. (2002). Pollen asthma and that treatment. *Allergy in Practice*, 22, 34–38. (in Japanese).
- Maejima, K., Tamura, K., Nakajima, T., Taniguchi, Y., Saito, S., & Takenaka, H. (2001). Effects of the inhalation of diesel exhaust, Kanto loam dust or diesel exhaust without particles on immune responses in mice exposed to Japanese cedar (*Cryptomeria japonica*) pollen. *Inhalation Toxicology*, 13, 1047–1063.
- Motta, A. C., Marliere, M., Peltre, G., Sterenberg, P. A., & Lacroix, G. (2006). Traffic-related air pollutants induce the release of allergen-containing cytoplasmic granules from grass pollen. *International Archives of Allergy and Immunology*, 139, 294–298.
- Nakamura, S., Sato, F., & Nakamura, N. (2004). Immunocytochemical localization of Cry j 1 and Cry j 2—the allergenic proteins of Japanese cedar pollen—in the germinated pollen. *Japanese Journal of Palynology*, 50(1), 15–22. (in Japanese).
- Namork, E., Johansen, B. V., & Løvik, M. (2006). Detection of allergens adsorbed to ambient air particles collected in four European cities. *Toxicology Letters*, 165, 71–78.
- Nishihata, S., Inoue, S., Saiga, T., Sahashi, N., Suzuki, S., Murayama, K., et al. (1999). Prevalence rate of allergy to Japanese cedar pollen in Tokyo. *The Japanese Society of Allergology*, 48(6), 597–604. (in Japanese).
- Okuda, M., Usami, A., & Kisikawa, R. (2006). Study of recent yearly trend of increase in airborne pollen of Japanese cedar and Cypress pollen. *The Japanese Society of Allergology*, 55(12), 1531–1535. (in Japanese).
- Okuyama, Y., Matsumoto, K., Okochi, H., & Igawa, M. (2007). Adsorption of air pollutants on the grain surface of

- Japanese cedar pollen. *Atmospheric Environment*, 41, 253–260.
- Rantio-Letimaki, A., & Matikainen, E. (2002). Pollen allergen reports help to understand pre-season symptoms. *Aerobiologia*, 18, 135–140.
- Rogerieux, F., Godfrin, D., Sènèchal, H., Motta, A. C., Marlière, M., Peltre, G., et al. (2007). Modifications of *Phleum pratense* grass pollen allergens following artificial exposure to gaseous air pollutants (O₃, NO₂, SO₂). *International Archives of Allergy and Immunology*, 143, 127–134.
- Sagehashi, M., Fukuda, T., Fujii, T., Sakai, Y., & Sakoda, A. (2005). Elution and adsorptive concentration of Japanese cedar (*Cryptomeria japonica*) pollen allergen in environmental water. *Water Science and Technology*, 52(9), 37–43.
- Sawatani, M., Ono, A., Kohno, K., Kawashima, T., Taniguchi, Y., Ikegami, H., et al. (1994). Enzyme-linked immunosorbent assay for the quantification of Cry j 1 and Cry j 2. *Japanese Society of Allergology*, 43(3), 467–473. (in Japanese).
- Schappi, G. F., Taylor, P. E., Staff, I. A., Rolland, J. M., & Suphioglu, C. (1999). Immunologic significance of respirable atmospheric starch granules containing major birch allergen Bet v 1. *Allergy*, 54(5), 478–483.
- Schappi, G. F., Taylor, P. E., Staff, I. A., Suphioglu, C., & Knox, R. B. (1997). Source of Bet v 1 loaded inhalable particles from birch revealed. *Sexual Plant Reproduction*, 10, 315–323.
- Suzuki, T., & Tsunogai, S. (1988). Origin of calcium in aerosols over the Western north pacific. *Journal of Atmospheric Chemistry*, 6, 363–374.
- Takahashi, Y., Ohashi, T., Nagoya, T., Sakaguchi, M., Yasueda, H., & Nitta, H. (2001). Possibility of real-time measurement of an airborne *Cryptomeria japonica* pollen allergen based on surface Plasmon resonance. *Aerobiologia*, 17, 313–318.
- Wang, Q., Gong, X., Nakamura, S., Kurihara, K., Suzuki, M., Sakamoto, K., et al. (2009). Air pollutant deposition effect and morphological change of *Cryptomeria japonica* pollen during its transport in urban and mountainous areas of Japan. *Environmental Health Risk V, Biomedicine and Health*, 14, 77–89.
- Wang, Q., Kurihara, K., Kiryu, H., Sakamoto, K., Miwa, M., & Uchiyama, I. (2008). Daily variation differences between airborne Japanese cedar pollen and its respirable allergen particulate matters during the most severe pollination seasons. *Eurozoru Kenkyu*, 23, 120–126. (in Japanese).