Contribution of airborne fine particles containing *Cryptomeria japonica* pollen allergens to airborne organic carbonaceous aerosols during a severe pollination episode

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Abstract

Japanese cedar pollinosis has been recognized recently as a serious social problem because of its high prevalence in Japan. It is well-known that the pollen grains of *Cryptomeria japonica* pollen (so-called Japanese cedar pollen) usually exist as coarse particles about 30 μm. However, it was supposed that the major allergen Cry j 1 (Cry j 1 particles) could be released to the atmosphere as respirable-sized particles and modified by some air pollutants during airborne transportation. Cry j 1 particles represent major seasonal allergen sources and are suspected to cause pollen asthma. Moreover, since Cry j 1 particles mainly consist of protein materials and cytoplasm from the pollens, they should be organic carbonaceous aerosols in fine particle sizes because protein materials are also some kind of organic carbon (OC). Therefore, one of the Cry j 1 release processes in which Cry j 1 eluted from several simulated rainfalls of various salt components have been investigated. As a result, about 60% of Cry j 1 was released in simulated rain containing Ca^{2+} ions. At the same time, it is important to examine the release behavior of Cry j 1 particles and to evaluate the source contributions calculated from Cry j 1 particles to organic carbonaceous aerosols. The aim of this study is to examine the particle size distribution of Cry j 1 and OC in airborne aerosols to clarify some mechanisms provoking pollen asthma and to evaluate source contributions during a severe pollination episode of FY 2005. Airborne Cry j 1 particles were collected with high volume Andersen air samplers, and Cry j 1 and OC (OC1-OC4) concentrations were determined by

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the ELISA method and the thermal-optical carbon analyzer respectively. More than 80% of Cry j 1 existed as fine particles below 1.1 μm, which consisted of OC2, OC3 and OC4. Source contributions of OC derived from pollen may be averagely occupied ~30% in airborne organic carbonaceous aerosols as total OC in fine particles ($PM_{1.1}$) below 1.1 μm respectively. Thus, it was possible that OC contribution below 1.1 μm is overestimated by conventional concepts of various anthropogenic and secondary formed sources because Cry j 1 particles are also below 1.1 μm which are the composite OC sources released from pollen grains.
Keywords: source contribution, $PM_{1.1}$, Japanese cedar pollen, allergen, Cry j 1, pollen asthma, organic carbonaceous aerosol, organic carbon (OC).

1 Introduction

In Japan, the prevalence of Japanese cedar (*Cryptomeria japonica*) pollinosis is reported to be about 20% and it can also be said to be the national illness (Shida [1]). The prevalence is increasing especially in urban areas where its incidence is about 28% (Bureau of Social Welfare and Public Health, Tokyo. [2]). Since the increase in the number of hay fever patients, the reduction in the age of development of symptoms, and the development of symptoms of asthmatic conditions (Maeda *et al*. [3]), especially in recent years, it is a big social problem as a disease, and an immediate remedy is needed.

The causative substances of Japanese cedar pollinosis are Japanese cedar pollen allergens, and there are two kinds of major allergens. One is the basic protein Cry j 1 of molecular weight *ca.* 40,000 which is localized Ubisch bodies (*ca.* 0.7 μm) attached mainly on the surface of Japanese cedar pollen (*ca.* 30 μm) (Yasueda *et al*. [4]). Another is called as Cry j 2 as the basic protein of molecular weight *ca.* 37,000 which is contained the starch granules and pollen lining membrane inside the pollen (Takahashi *et al*. [5]).

Furthermore, although it had been thought that *Cryptomeria japonica* pollen invaded only the human nasal cavity and mouth, and there was originally no inhalation to a lower respiratory tract, existence of the airborne suspended particulate matter containing the *Cryptomeria japonica* pollen allergens (Cry j 1 particles or Cry j 2 particles) which can invade into a lower respiratory tract has now been suggested. Some researchers found and reported that respirable allergen particles are exfoliated Ubisch bodies from the pollen surface or starch granules from ruptured pollens (Kurihara *et al*. [6] and Wang *et al.* [7]). Also respirable allergen particles invade into a lower respiratory tract and develop pollen asthma. It is very important to investigate the *Cryptomeria japonica* pollen main part in the urban atmosphere and the airborne behavior of allergen particles, and to grasp the dynamic state, because we want to evaluate the influence of allergen particles in the development of pollinosis, pollen asthma and the modification of allergen particles with air pollutants. In addition, long range transportation phenomena of yellow sand from the East Asian continent were found during the pollen scattering season in Japan due to the global warming. Therefore, interaction between pollen and yellow sand should be of

concern. Actually, the pollinosis was enhanced when pollen and crustal particles (Maejima *et al*. [8]) were inhaled at the same time.

Since Cry j 1 particles mainly consist of protein materials and cytoplasm from the pollens, they should be classified into organic carbonaceous aerosols from a viewpoint of the environmental aerosol chemistry because protein materials are also some kinds of organic carbon (OC). Moreover, most of Cry j 1 particles exist in the fine particle ranges based on our study on the size distribution of Cry j 1 (Wang *et al.* [7]). It is well known that OC component contained in the suspended particulate matter (SPM) of the fine particle range are mainly conventionally considered to be the particles caused from secondary formation and the anthropogenic sources. It is considered to be important how particulate matters from the natural sources take into consideration about the emission sources of the fine particles to grasp the level of the influences (contributing ratio) of the pollen allergen containing particles as a primary emission and the natural sources. Therefore, for new knowledge and a new view advocated about the emission sources of OC fine particles, especially in research of a severe pollination episode, it is necessary to grasp the OC contribution of the particles containing pollen allergen particles as a primary emission and the natural sources during a severe pollination episode.

In this paper, firstly, in order to check that the major allergen Cry j 1 particles exist in the fine particle ranges, the collection classified by particle diameters of the airborne *Cryptomeria japonica* pollen of a pollination season was performed, and the size distribution of Cry j 1 in SPM was investigated by measuring Cry j 1 by the analyzing method using the antibody which recognizes specifically Cry j 1. Moreover, OC concentration in the SPM of a pollination season and Cry j 1 concentration were measured according to particle diameter, and the OC concentration measurement (or contribution ratio) of the particles of the natural pollen source in the fine particles were estimated by computing and evaluating the contribution in the fine particles based on the results of OC composition measured from Cry j 1 standard samples.

2 Experiment methods

2.1 Confirming one transition process of Cry j 1 to fine particle sizes when contacted rainfall

To confirm transition of Cry j 1 to fine particle sizes when contacted rainfall, 500 mg pollen was mixed with various solutions of inorganic salts, ultra pure water and extraction solution. Extraction solution was usually prepared for effective elution of Cry j 1 from pollen grains (Kurihara *et al.* [6]). These inorganic concentrations of KNO_3, NH_4NO_3 (secondary particles) and $Ca(NO_3)_2$ (derive from yellow sand) were 80, 150, 500 and 1500 mN, respectively. The *Cryptomeria japonica* pollen solutions were made from eluted allergen contents by filtration using a cellulose acetate membrane filter (pore size is 1.2 μm). And, pollen solutions were treated after centrifuge separation (15,000 rpm, 10 min). Supernatant liquids were picked 1 mL, and it was exchanged to buffer solution (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20) by a

centrifugal filter unit (amiconultra-4, Millipore). Then, Cry j 1 concentrations were measured by the surface plasmon resonance method (Model Biacore J system, GE Healthcare Co. Ltd).

2.2 Sampling sites, periods and collection of air samples

Airborne *Cryptomeria japonica* pollens and allergen particles were collected at the mountain and urban areas of Saitama in Kanto area, Japan. (1) Mountain area: Air samples in three different sizes (< 1.1 μm, 3.3–7.0 μm, > 7.0 μm) were collected on tissue-quartz filters using high volume air samplers (Shibata Co. Ltd., AH-600, AHV) with a flow rate of 566 L/min for the 23 hours sampling from March 29 to April 4, 2005. (2) Urban area: Air samples were collected using an AHV with a flow rate of 566 L/min for the 23 hours sampling from March 10 to March 16, the severe pollination episode of FY2005.

2.3 Extraction procedure of pollen allergen (Cry j 1) from air samples

The tissue-quartz filters (8 mmϕ) collected air samples were cut out and put into centrifuge tubes (Polypropylene copolymer; PPCO), and then they were filled with Cry j 1 extract (0.125 M NH_4HCO_3 solution containing 150 mM NaCl, 3 mM EDTA, 0.005 wt % Tween 20, and 10 mM HEPES buffer solution) 2 mL (Kurihara *et al*. [6]). After carrying out the standing of the solutions in 4 degrees Celsius for 24 hours, the shaking was carried out at the shaking velocity of 192 rpm at the room temperature for 1 hour (Heidolph Co. Ltd., UNIMAX 2010). Then, the centrifugation was carried out by 3000 rpm for 30 minutes at the room temperature (Kokusan Co. Ltd., H-11NA), and the supernatant solutions were prepared as the samples for the pollen allergen Cry j 1 determination.

2.4 Determination of pollen allergen (Cry j 1) concentrations

Cry j 1 concentrations in the samples were quantified by an ELISA method using anti-Cry j 1 monoclonal antibody (Seikagaku Biobusiness Co. Ltd, clone 013) and peroxidase conjugated anti-Cry j 1 antibody (Seikagaku Biobusiness Co. Ltd, clone 053) which is one of the used enzyme immunoassay, so it is high sensitivity and alternative measuring. Coloring substrate is *o*-phenylenediamine, measurement absorption wavelength of a micro plate leader (Microtech Co. Ltd, MP-1000) is 492 nm. Finally, the absorbance of each solution obtained by an ELISA method were converted into Cry j 1 concentrations (ng/m^3) in the atmosphere.

2.5 Calculation method of OC contributing ratio to SPM derived from Cry j 1 particles

The OC contribution ratios to SPM estimated from Cry j 1 particles was computed as the following equation (1).

$$\text{Contribution ratio}\ (\%) = \frac{\text{OC conc. from Cry j 1 particles}}{\text{OC conc. from SPM}} \times 100 \qquad (1)$$

Here, OC concentrations from Cry j 1 particles are the values of the OC concentration derived from the measurement results of Cry j 1 concentration in the tissue-quartz filters collected air samples (*i.e.* SPM). OC concentrations from Cry j 1 particles can be calculated according to equation (2).

$$\text{OC conc. from Cry j 1 particles} = \frac{\text{Cry j 1 conc. from SPM} \times \text{OC mass of standard sample}}{\text{Cry j 1 mass of standard sample} \times \left(\frac{\text{Cry j 1 conc.}}{\text{Pollen mass}} \right)} \quad (2)$$

In addition, OC mass of standard sample and Cry j 1 mass of standard sample in equation (2) were obtained by measuring the concentration of OC component contained in the Cry j 1 standard samples produced from the refining *Cryptomeria japonica* pollen antigen Cry j 1 of commercial elegance (the details of production of a Cry j 1 standard sample and the analysis procedure of OC component are indicated in section 2.5. Moreover, Cry j 1 concentration of pollen is 10 ng for each pollen obtained from the literature (Yasueda *et al*. [9]), and the pollen in mass is 4.2 pg for each (Saito *et al.* [10]).

2.6 Quantitative analysis of carbon components contained in SPM in air samples and in Cry j 1 standard samples

In order to obtain OC concentration in Cry j 1 standard samples refined *Cryptomeria japonica* pollen antigen Cry j 1 10 μL dropped on a quartz fiber filter (6 mmϕ). After it was dried (25 degrees Celsius), it was used as Cry j 1 standard samples. It measured according to the carbon components of (OC1–OC4, EC1–EC3 based on their measurable temperatures) by a thermal optical carbon analyzer (Atmoslytic Co. Ltd, DRI Model 2001 OC/EC Carbon Analyzer) of IMPROVE method. The analysis conditions of this thermal optical carbon analyzer are given in table 1. In addition, OC1 are apparently volatile organic carbon below 120 degrees Celsius which can be negligible in our analytical results because pollen allergens are consisted with non-volatile protein materials.

Table 1: Analysis conditions of carbon concentrations of air samples.

Carbon fraction	Flow gas	Temperature (degrees Celsius)
OC1	100% He	~120
OC2		120~250
OC3		250~450
OC4		450~550
EC1	98 % He 2 % O_2	~550
EC2		550~700
EC3		700~800

Next, quantitative analysis of the carbon components in the quartz fibre filters (6 mmϕ) which collected SPM in air samples carried out in the same procedure as analysis of in the Cry j 1 standard samples. The contributing ratio was calculated by measuring the concentration of an OC concentration according to particle sizes in SPM.

3 Results and discussion

3.1 Transition concentrations to fine particle sizes and behavior of Cry j 1 in simulated rain solution of different cations

Cry j 1 concentrations which transition to fine particle sizes from *Cryptomeria japonica* pollen grains in the simulated rain solutions of different cations (K^+, NH_4^+ and Ca^{2+}) and extraction solution were shown in **figure 1**. In this result, higher concentrations of Cry j 1 were eluted in $Ca(NO_3)_2$ simulated rain than the others.

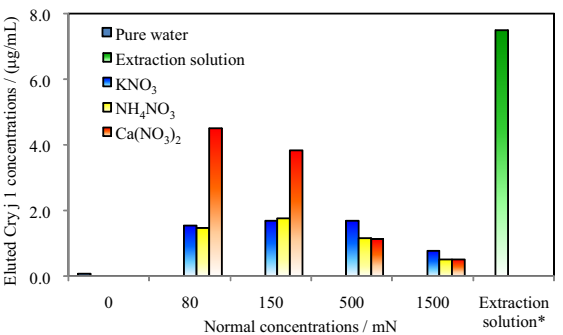

Figure 1: Tendency of eluted Cry j 1 concentrations in various normal concentrations and salt components. These samples were filtered by filter of pore size 1.2 μm.
*In this solution, Cry j 1 is eluted from *Cryptomeria japonica* pollen grains effectively.

On the other hand, eluted concentrations were decrease when the simulated rain contained less than 80 mN or more than 500 mN in the concentrations. There were no differences in eluted Cry j 1 concentrations between the normal concentrations of 80 and 150 mN of the same salt, especially for a Ca^{2+} rich solution. However, they were reduced in proportion to inorganic normal concentrations between the concentrations of 150 and 1500 mN. And about 60% Cry j 1 was eluted in $Ca(NO_3)_2$ simulated rain compared with extraction solution. We guess that *Cryptomeria japonica* pollen grain has a cell membrane like a semipermeable membrane and it will be ruptured by an expansion with osmotic pressure by various ionic normal concentrations of inorganic salts in rainfall.

3.2 Size distribution and possible airborne behaviour of Cry j 1 particles

The size distribution of Cry j 1 in different particle sizes and average airborne *Cryptomeria japonica* pollen counts were shown in **figure 2** and Cry j 1 concentrations in filters (below 1.1 μm) was shown in **figure 3**. In figure 2, Cry j 1 particles were most detected at the below 1.1 μm in all sampling periods. It is obvious that more than 80% of Cry j 1 concentration of all particle ranges existed in fine particles ($PM_{1.1}$) below 1.1 μm which are respirable-sized particles.

The particle sizes of *Cryptomeria japonica* pollen is about 30 μm but the Cry j 1 particles may be released to the atmosphere when Ubisch bodies are exfoliated from pollen surfaces and Cry j 1 contents are also eluted from pollens by contacting rainfall. Therefore, Cry j 1 content shifts to the fine particle sizes as the respirable-sized particles.

Moreover, exfoliated Ubisch bodies of *Cryptomeria japonica* pollen grains in an air samples were observed by a scanning electron microscope (SEM) like proving this (figure 4(a)). And ruptured pollen of content (starch granule, Golgi body, cytoplasm etc.) inside pollen which contains Cry j 1 and Cry j 2 were observed by a SEM (figure 4(b)). In our laboratory, we checked that Japanese cedar pollen allergen Cry j 1 contents are eluted by contacting the rain water, so Cry j 1 particles are possible to release from exfoliated Ubisch bodies of *Cryptomeria japonica* pollen grains, released pollen content and eluted Cry j 1 by rain and so on. Since the major allergen Cry j 1 particles can be released to the atmosphere as respirable-sized particles, and the size distribution is even similar with the particles emitted from some anthropogenic sources such as diesel exhaust particles (DEP) (Jiang *et al.* [11]) and secondary formation particles, it was supposed that these pollen allergen particles may be modified by the combination and/or reaction with some air pollutants during airborne transportation.

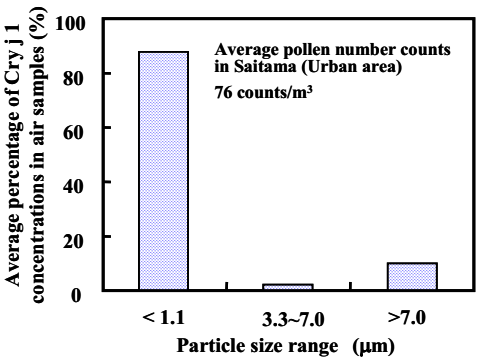

Figure 2: Average size distribution of Cry j 1 concentrations in air samples of <1.1, 3.3–7.0, >7.0 μm in Saitama (Urban area) and average airborne pollen number counts.

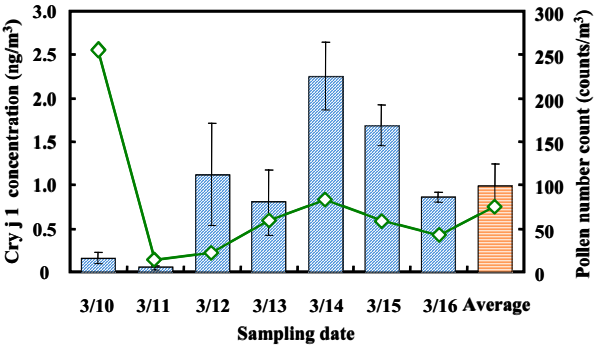

Figure 3: Cry j 1 concentrations in $PM_{1.1}$ and *Cryptomeria japonica* pollen number counts for each sampling period in Saitama (Urban area).

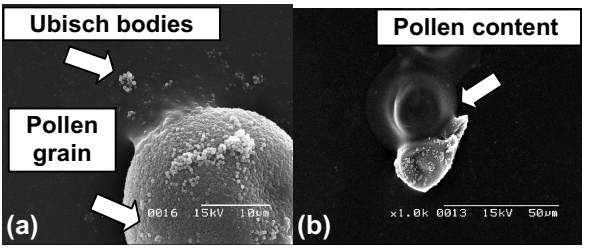

Figure 4: Scanning electron micrographs of airborne pollen. (a) Exfoliation of Ubisch body (b) Pollen rupture and release of *Cryptomeria japonica* pollen grains.

3.3 Composition analysis of carbonaceous components in the Cry j 1 standard samples

The concentrations of the various carbonaceous components in the Cry j 1 standard samples were shown in **table 2**.

Table 2: Carbonaceous concentrations in Cry j 1 standard samples and the percentages of them.

Carbon fraction	Carbon concentrations (μg/cm^2)	Percentage of carbon concentrations (%)
OC1	0.09	8.7
OC2	0.08	7.8
OC3	0.60	58.3
OC4	0.26	25.2
EC1	0.00	0.0
EC2	0.00	0.0
EC3	0.00	0.0

As the results of the carbonaceous measurements, we found about 58% of Cry j 1 consists of OC3 and about 25% consists of OC4. Since its molecular weight about 40,000, it is considered to be measured in OC3 and OC4 with the high determining temperatures above 250 degree Celsius. Therefore, OC contributing ratio to SPM derived from Cry j 1 particles is also manly computed and evaluated by the OC results of OC3 and OC4.

3.4 Investigation of OC contribution ratios to SPM derived from Cry j 1 particles in air samples

The concentrations of OC3 and OC4 in particles below 1.1 μm *i.e.* $PM_{1.1}$ were shown figure 5 and figure 6. Contribution ratios of OC3 and OC4 to $PM_{1.1}$ estimated from the OC3 and OC4 caused from Cry j 1 particles was calculated using equation (1) and equation (2). In addition, the values of (OC3 mass / Cry j 1 mass) and (OC4 mass / Cry j 1 mass) are 0.17 ±0.01, 0.074±0.01 respectively by carbonaceous analysis in the Cry j 1 standard samples.

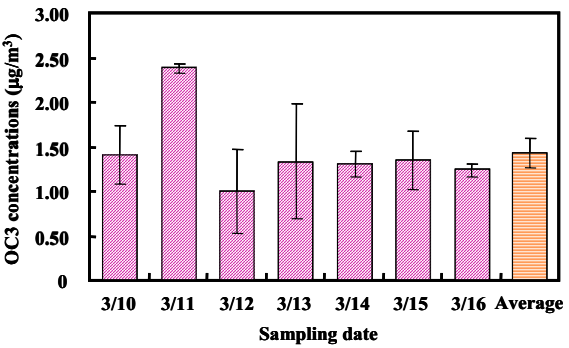

Figure 5: OC3 concentrations in air samples of < 1.1 μm in Saitama (Urban area).

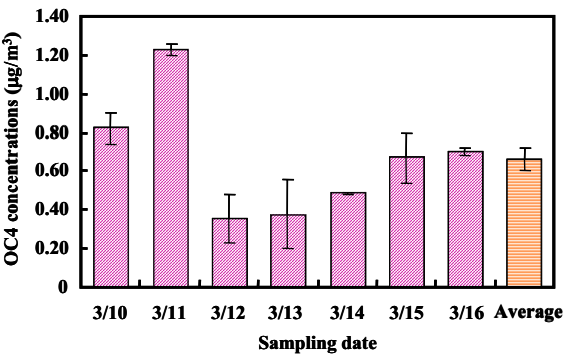

Figure 6: OC4 concentrations in air samples of < 1.1 μm in Saitama (Urban area).

The Contribution ratios to OC2, OC3 and OC4 in $PM_{1.1}$ are shown in table 3. In the severe pollination episode of FY2005, the average Contribution ratio to OC2 was 3.3%, the ratio to OC3 was 13% and the ratio to OC4 was 15% in $PM_{1.1}$. It is thought conventionally that the aerosols from primary emission and natural sources such as pollen have the very low OC contribution to $PM_{1.1}$ since they distributed over the coarse particle sizes. However, Cry j 1 particles from exfoliated Ubisch bodies and eluted Cry j 1 from pollen grains were estimated that they contributed to OC2, OC3 and OC4 in the fine particle sizes. Especially, OC of Cry j 1 particles contribute to OC2, OC3 and OC4, less volatile influence to OC1 and Cry j 1 has hydrophilic and water solubility, so it is guessed the Cry j 1 particles may also play a role of the nuclear ambient aerosols.

Table 3: Contribution ratios of OC derived from Cry j 1 particle to ambient OC in $PM_{1.1}$ in the sampling periods.

Sampling period	Contributing ratios* (%)		
	OC2	OC3	OC4
3/10	0.30 ±0.10	1.9 ±0.71	1.5 ±0.53
3/11	0.050 ±0.017	0.36 ±0.17	0.31 ±0.15
3/12	7.0 ±2.4	19 ±10	23 ±12
3/13	3.6 ±1.2	10 ±4.8	16 ±7.5
3/14	7.0 ±2.4	29 ±5.0	34 ±5.9
3/15	3.7 ±1.3	21 ±2.9	19 ±2.5
3/16	1.5 ±0.52	12 ±0.80	9.1 ±0.62
Average	3.3 ±1.1	13 ±2.9	15 ±2.8

*Contributing ratio of OC derived from pollen to ambient OC.
– means concentrations in air samples are below concentrations in blank sample.

4 Conclusion

In this study, we confirmed that Cry j 1 particles were transferred to fine particle sizes when contacted rainfall as the composite OC sources released from pollen grains. The air sampling of different particle sizes of the airborne pollen and allergen particles was performed during a severe pollination season of FY2005. As a result, more than 80% of Cry j 1 particles were detected as the fine particles ($PM_{1.1}$) below 1.1 μm, and it is clear that allergen Cry j 1 particles will release from *Cryptomeria japonica* pollen grains during their transportation.

Then, we measured and calculated the OC contributing ratios to SPM especially for $PM_{1.1}$ derived from Cry j 1 contents in air samples. Their contribution to ambient aerosols as the natural source particles was firstly reported. In the collection period, the average contributing ratio to OC2 was 3.3%, the ratio to OC3 was 13% and the ratio to OC4 was 15% in $PM_{1.1}$. Since

the contribution ratios showed the rate of OC contents of the Cry j 1 particles to OC concentration in $PM_{1.1}$, it can be estimated that Cry j 1 particles were occupied about 30% (averagely at the maximum) among total OC concentrations of OC2, OC3 and OC4 of the fine particles during a severe pollination season. However, actually, we prepared various simulated rain waters for the Cry j 1 elution experiment, because extraction solution was not existed in natural atmosphere. Cry j 1 in simulated rain contained Ca^{2+} derived from yellow sand was eluted 60% compared with extraction solution. Therefore, OC contribution ratio of Cry j 1 was possible overestimation, and this result indicated that yellow sand is one of the influence factors on release and transition of Cry j 1 to fine particle sizes. Nevertheless, it is the concentration level which cannot be negligible and the contributions have to be divided from those of secondary formation and the other anthropogenic sources.

In addition, since the concentrations were varied sharply day by day, the continuous investigation which associated the concentration of OC in the releasing factor of Cry j 1 particles such as rainfall and that in the fine particles is required.

Moreover, from this study, it is obvious that *Cryptomeria japonica* pollen allergen Cry j 1 particles exist as fine ones below 1.1 μm. However, as for other pollen allergen, the possibility of existing even in the size order of nanometer is reported (Taylor *et al.* [12]). Some of Cry j 1 particles may also similarly exist as the particles of nanometer order. In this case, since the residence time of Cry j 1 nano-particles in the atmosphere becomes long rather than the fine particles.

Finally, we should consider that measuring and evaluating of seasonal pollen allergen particles as their hydrophilic nucleation of ambient aerosols on the local climate effects (Ortiz *et al.* [13]) and their modification with the air pollutants are acquired. Therefore, it will be necessary to investigate the propagation reactions, primary emission sources of natural aerosols and the size distribution of the other seasonal pollen allergen particles in the atmosphere during their severe pollination episodes.

Acknowledgements

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