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Size-segregated Allergenic Particles Released from Airborne *Cryptomeria japonica* Pollen Grains during the Yellow Sand Events within the Pollen Scattering Seasons

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ABSTRACT

Cryptomeria japonica pollen is the most common pollen, which are scattering during each spring season in Japan. Japanese cedar (*Cryptomeria japonica*) pollinosis is one of seasonal allergic rhinitis that mainly occurs in Japan. In addition, long range transportation of Yellow Sand from the East Asian continent was also found during the pollen scattering seasons in Japan. Therefore, the interaction or impact between pollen and Yellow Sand should be concerned. In this study, our objective was to investigate the airborne behaviour of *Cryptomeria japonica* pollen grains and its size-segregated allergenic (Cry j 1) particles as the airborne tracer of *Cryptomeria japonica* pollen during the Yellow Sand events. Airborne *Cryptomeria japonica* pollen grains and its size-segregated allergenic particles were collected at roadside of urban residential zones of Saitama city during the pollination periods from February to March in two year investigation of 2009 and 2010. The overlap of Yellow Sand events and dispersal peak of pollen grains was observed. According to the Meteorological data, we found that the peaks of airborne pollen grains appeared under higher wind speed and temperature than the previous day. It was thought that Yellow Sand events and airborne pollen counts were related to wind speed. From the investigation of the airborne behavior of the size-segregated allergen particles by determining Cry j 1 with Surface Plasmon Resonance (SPR), the higher concentrations of the allergenic Cry j 1 were detected in particle size equal to or less than 1.1 μm ($PM_{1.1}$) than other particle sizes during Yellow Sand events, especially in the rainy day. We conclude that rainwater trapping Yellow Sand is one of the important factors that affect the release of allergenic pollen species of Cry j 1. Therefore, it is very important to clarify the relationships between *Cryptomeria japonica* pollen allergenic species and chemical contents of the Yellow Sand particles in further studies.

Key words: Japanese cedar pollinosis, *Cryptomeria japonica* pollen, Size-segregated Allergenic Species, Yellow Sand events, Surface Plasmon Resonance (SPR).

1. INTRODUCTION

Allergic rhinitis is increasing day by day both in prevalence and severity in most of the countries which is affecting 25% of the population worldwide. Pollen is the most important reported cause of seasonal allergic rhinitis (Zhang *et al.*, 2012). In Japan, main seasonal allergic rhinitis is Japanese cedar (*Cryptomeria japonica*) pollinosis, which has been increased during the past decades. In 2008, the average prevalence of Japanese cedar pollinosis is 26.5% in Japan, although there are great regional differences in the prevalence of this disease (Murayama *et al.*, 2010). The alarming numbers of the patients suffer from symptoms, such as rhinorrhea, nasal obstruction, and nasal itching, sneezing, and itching of the eyes due to pollinosis (Tanaka *et al.*, 2012). The increment of the airborne *Cryptomeria japonica* pollen counts is the main nominated reason, while many other factors such as changes in the urban environmental pollution and human lifestyle are pointed out as well. *Cryptomeria japonica* forests cover nearly 18% of the total land area of Japan, concentrated in the Kanto region and western part of the country (Yonekura *et al.*, 2012). *Cryptomeria japonica* pollen can be dispersed to long distance like several tens of kilometers by wind. According to these research results in urban cities of Kanto plain, there are many patients in urban areas due to dispersion of pollen to long distance. The pollinosis is one of the typical allergic disease caused by two main kinds of allergens called Cry j 1 and Cry j 2 which are exist in *Cryptomeria japonica* pollen. The allergenic Cry j 1 particle is a basic protein with molecular weight of 41 kDa and 44 kDa, exists to pollen wall and in Ubisch body. The allergenic Cry j 2 particle is also a basic protein with molecular weight of 37 kDa, exists to starch granule in pollen grain and its content is tenth of Cry j 1 (Nakamura *et al.*, 2004). It had been considered that the pollen in coarse particles about 30 μm only deposits in upper part of the respiratory system such as human nasal cavity and mouth, and originally there was no inhalation to lower respiratory tract. However, the existence of airborne fine particles such as the particle sizes below 1.1 μm ($PM_{1.1}$) containing the *Cryptomeria japonica* pollen allergen particle (Cry j 1) had been confirmed in our previous study (Wang *et al.*, 2008).

In addition, the Yellow Sand (Asian-dust) phenomenon occurs in springtime, and has been well known as a seasonal tradition since time immemorial in Japan. The main cause is severe dust storms occurring in connection with a low-pressure system that develops from March to May in arid and semi-arid regions of northeast Asia, particularly in the Taklimakan Desert, Gobi Desert, and Loess Plateau regions in China and Mongolia (Shao *et al.*, 2006). In recent years, characteristic Yellow Sand events were also observed in East Asia, and the peak of *Cryptomeria japonica* pollen scattering had also appeared at the same period in Japan. The seasonal overlap of *Cryptomeria japonica* pollen dispersal and Yellow Sand events occurrence may also have an impact on allergic rhinitis in Japanese patients. Watanabe *et al.* (2011) studied Yellow Sand influence on Japanese adult patients with asthma by conducting a telephone survey, finding that 11%-22% of patients noted worsening lower respiratory symptoms during Yellow Sand events increased when *Cryptomeria japonica* pollen was present simultaneously in the atmosphere. The silicon dioxide and elements originated from microorganism that adheres to Yellow Sand might be a cause of ingravescence of an allergy to *Cryptomeria japonica* pollen by the experiment using a guinea pig (Ichinose, 2011). Therefore, interaction or impact between pollen and the Yellow Sand events should be concerned.

In this study, we focused on the airborne tracer of *Cryptomeria japonica* pollen grains and its size-segregated allergenic particles (mainly Cry j 1) located to the *Cryptomeria japonica* pollen surface during the Yellow Sand events within the pollen scattering seasons in 2009 and 2010. From the investigation of the airborne behavior of the size-segregated allergen particles by determining Cry j 1 during the Yellow Sand events, we may figure out the relationships between allergenic release behavior from *Cryptomeria japonica* pollen and Yellow Sand.

2. EXPERIMENTAL DESIGN

2. 1 Sampling for Atmospheric Particles

In order to grasp the airborne behavior of *Cryptomeria japonica* pollen grains and its size-segregated allergen particles (Cry j 1) during the Yellow Sand events, we collected atmospheric particles within the pollen scattering seasons. Our sampling site was located at the roadside of Saitama University which is the urban residential zone of Saitama City in the north of Tokyo metropolitan area, Japan (Fig. 1).

All the air samples collected on the quartz fiber filters by Andersen high-volume sampler (AH-600, Shibata Scientific Technology Co., Ltd., Japan). The instrument collected the atmospheric suspended particulate matter equivalent to the human respiratory system as five size-segregated particles ($\leq$1.1, 1.1~2.0, 2.0~3.3, 3.3~7.0, $\geq$7.0 μm). Because pollen allergens were confirmed abundantly in fine particles $\leq$1.1 μm ($PM_{1.1}$) and coarse particles $\geq$7.0 μm ($PM_{\geq 7.0}$) in our previous studies (Wang *et al.*, 2012b; Wang *et al.*, 2008), we only measured the pollen allergenic Cry j 1 concentrations in particle sizes of $PM_{1.1}$ and $PM_{\geq 7.0}$ for finding the airborne behavior of the allergenic particles during the Yellow Sand events. Air flow of the sampler was 566 L/min. The filters were changed each 24 hours (March 9 and March 10, 2009), each 47 hours (March 11-20, 2009) and each 71 hours (March 11-28, 2010).

In order to count pollen grains in atmosphere, airborne pollen grains were collected by automatic pollen monitor KH-3000-01 (Yamatronics Co., Ltd., Japan). Automatic pollen monitor KH-3000-01 is automatic meter reading device, which uses semiconductor laser for light source, forward and sideways-scattered pulses induced by each particle, calculates automatically the number of equivalent to 28-35 μm particles. Automatic pollen monitor KH-3000-01 calculates the pollen counts within every 30 minutes at a flow rate 4.1 L/min, the results were converted into the average counts of the pollen counts of 30 minutes from 1.0 m^3 volume (counts/m^3/30 min).

In order to confirm the accuracy of the automatic pollen monitor KH-3000-01 during the Yellow Sand events, airborne pollens were also collected on a glass slide coated white petrolatum by the Durham sampler at the same time (DK-1SA, Nishiseiki Co., Ltd., Japan)

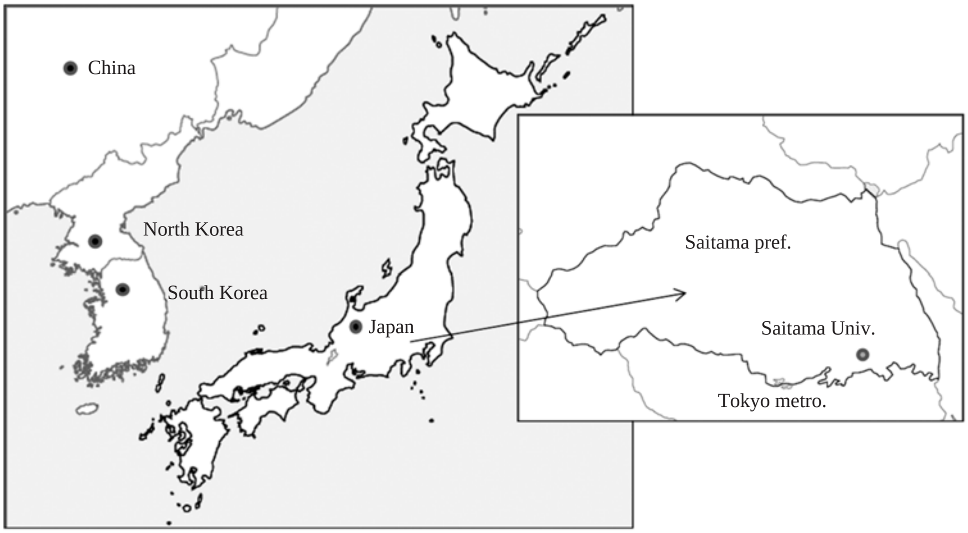

Fig. 1. Sampling site.

for each 23 hours too.

We have used the average pollen counts for each day calculated with the pollen counts of each 30 min interval during 24 h using the automatic pollen monitor KH-3000-01. However, we can only measure the pollen counts manually with an optical microscope for each 24 h interval, which is daily basis.

2. 2 Measurement Methods of Allergenic Cry j 1 Concentrations

The allergenic Cry j 1 particle was extracted using following method. The filters that collected from airborne particles in 2009 and 2010 were cut out (8 mmϕ, 30 pieces) and put into centrifuge tubes (polypropylene copolymer), followed by the addition of 2 mL of pollen extracting solution (0.125 M NH_4HCO_3, 150 mM NaCl, 3 mM EDTA, 0.005 wt% Tween 20, and 10 mM HEPES buffer solution) (Takahashi *et al.*, 2002; Takahashi *et al.*, 2001). After incubation of 24 hours at 4°C in the incubator (UNIMAX 2010, 25 Heidolph Co. Ltd.), the centrifuge tubes were shaken at 192 rpm at room temperature for 1 hour by vibrator, followed by centrifugation at 6000 rpm for 30 minutes at room temperature using the centrifuge (CN-1050, AS ONE Corp., Japan). The supernatant were collected and used for analysis.

We determined Cry j 1 concentrations by using a Biacore J system (GE Healthcare Japan Co., Ltd., Japan) based on surface plasmon resonance (SPR) principle. As anti-Cry j 1 monoclonal antibody (MAb, clone 013, Asahi Food & Healthcare Co., Ltd.) are immobilized on the surface of CM5 sensor chip (GE Healthcare Co., Ltd.), the refractive index at the interface between the sensor chip surface and solution flowing over the changes surface, altering the angle at which reduced-intensity of polarized light is reflected from a supporting glass plane. The angle change, caused by binding or dissociation of anti-Cry j 1 monoclonal antibody from the sensor chip surface, is proportional to the mass of bound material which is recorded in sensor gram. When solution containing Cry j 1 antigen is passed over the sensor chip surface, the sensor gram shows an increasing response with molecules interact. The response remains constant if the interaction reaches equilibrium. When solution containing Cry j 1 antigen is replaced by buffer, the response decreases as the interaction partners dissociate. Complete profiles of recognition, binding and dissociation are generated in real time. Finally, the concentration of Cry j 1 (ng/m^3) in atmosphere can be calculated (Wang *et al.*, 2012a).

2. 3 The Information of Yellow Sand Events and Meteorological Data

The Information of scattering of Yellow Sand events in the Kanto region was referenced from web site of the Ministry of Environment (http://www.data.kishou.go.jp/obs-env/kosateikyou/kosa.html). The Ministry of Environment is using LIDAR (Light Detection and Ranging) to observation of Yellow Sand. LIDAR is a remote sensing technology that can distinguish Yellow Sand particles which cannot be seen with the naked eye from other atmospheric pollutant particles, and such particles can be monitored on a real-time basis. In this page (http://soramame.taiki.go.jp/dss/kosa/), the concentration of Yellow Sand near the ground is estimated and represented as cylinders based on the monitoring results (provisional data) of LIDAR.

Meteorological data (wind speed, wind direction, temperature and precipitation) were obtained from the Saitama Institute of Public Health and the Okubo puification plant, located about 1.0 km from the sampling site.

3. RESULTS AND DISCUSSION

3. 1 Behavior of Pollen Grains during the Yellow Sand Events

The number of *Cryptomeria japonica* pollen grains was measured by KH-3000-01 and the dispersion of Yellow Sand events in 2009(a) and 2010(b) were showed in Fig. 2. Fig. 2a indicates that the volume of scattering pollen reached at the peak on March 10-12 and March 16-20, 2009. According to the official data from the Ministry of Environment (http://www.data.kishou.go.jp/obs-env/kosateikyou/kosa.html), wide range of Yellow Sand events and overlapping of Yellow Sand events peak and dispersal peak of pollen grains was observed during this period.

In 2010, airborne behavior of pollen grains during the Yellow Sand events was investigated as well. According to the Ministry of Environment, wide range of Yellow Sand events was observed on March 20-21, 2010. The number of scattering pollen grains reached the peak on March 21, and the overlap of Yellow Sand events peak and pollen dispersal peak were observed again (Fig. 2b). It turned out that the Yellow Sand events term and the *Cryptomeria japonica* pollen term overlapped.

The pollen counting device KH-3000-01 specialized in the detection of pollen particles in 28-35 μm diameters. Although the size of Yellow Sand particle which flies to Japan is near 4 μm (The report of Ministry of Environment, 2009), it was thought that Yellow Sand and pollen grains might not be distinguished completely by KH-3000-01. In order to determine the tendency of the pollen counts using the automatic pollen monitor KH-3000-01 during the Yellow Sand events, airborne pollen grains were also collected on a glass slide coated white petrolatum by the Durham pollen sampler at the same time. Durham method is a widely used gravitational method which distinguishes pollen grains from Yellow Sand. The pollen counts of the automatic pollen monitor KH-3000-01 seem to be well correlated with those of the Durham sampler ($R=0.89$, $n=7$, (2009); $R=0.86$, $n=7$, (2010)) which was showed in Fig. 3. Therefore, the KH-3000-01 automatic pollen monitor with the intensities of two directions of scattering of semiconductor laser light could be used for easier evaluation of the tendency of the pollen counts

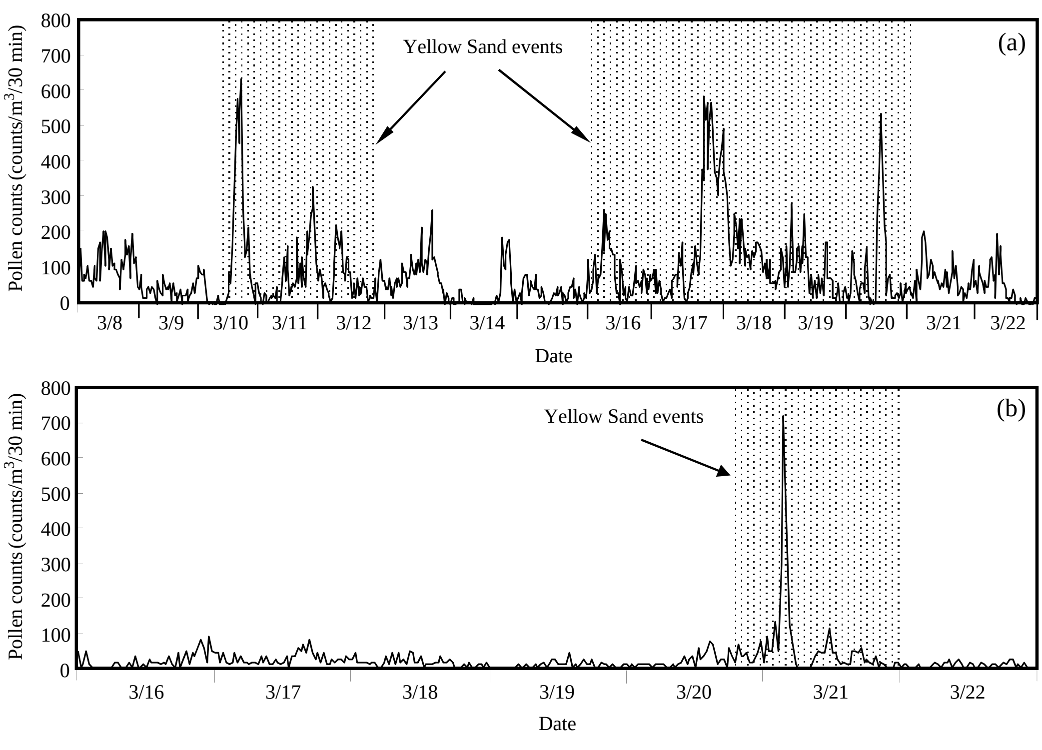

Fig. 2. Pollen counts by automatic pollen monitor KH-3000-01 and the flying information of the Yellow Sand events during Mar. 8-22nd, 2009 (a) and Mar. 16-22nd, 2010 (b).

than that of the Durham pollen sampler.

Fig. 4 shows the daily variation of wind speed, temperature and precipitation during the pollen scattering spring seasons in 2009 (a) and 2010 (b). Peaks of airborne pollen grains appeared on March 10, 17 and 20 under higher wind speed and temperature (Fig. 4a). Fig. 4b shows that peaks of airborne pollen grains appeared

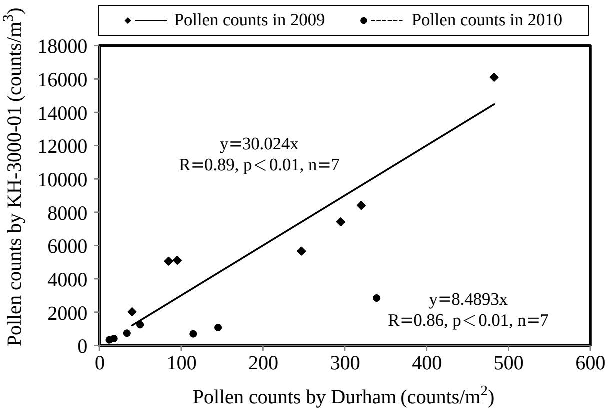

Fig. 3. Comparing pollen counts between by automatic pollen monitor KH-3000-01 and by Durham sampler.

on March 21 under higher wind speed and temperature than the previous day. Tamura *et al.* (1997) concluded that the highest temperature was statistically related to the maximum pollen counts of Japanese cedar in the air. The wind speed shown in Fig. 4 (March 10, 17 and 20 in 2009, March 21 in 2010) was higher than other days during Yellow Sand events. It was thought that strong wind speed in local scale may accelerate release of the pollen grains. Moreover, long-distance transportation of the pollen grains from mountains to the sampling site may also occur with higher wind speed.

3. 2 Airborne Behavior of Size-segregated Allergen Particles before and after Rainfall

According to the meteorological data of precipitation (mm) (Fig. 4), we found that on March 10th (5.5 mm), 14th (4.5 mm), 20th (1 mm) and 22nd (3.5 mm), 2009 and on March 10th (9.5 mm), 16th (10.5 mm), 21st (1 mm), 23rd (1.5 mm), 24th (23.5 mm), 25th (19 mm), and 26th (1.5 mm), 2010 had rained. Fig. 5 indicated the size-segregated allergenic Cry j 1 concentrations in 2009 (a) and 2010 (b). We noticed that although

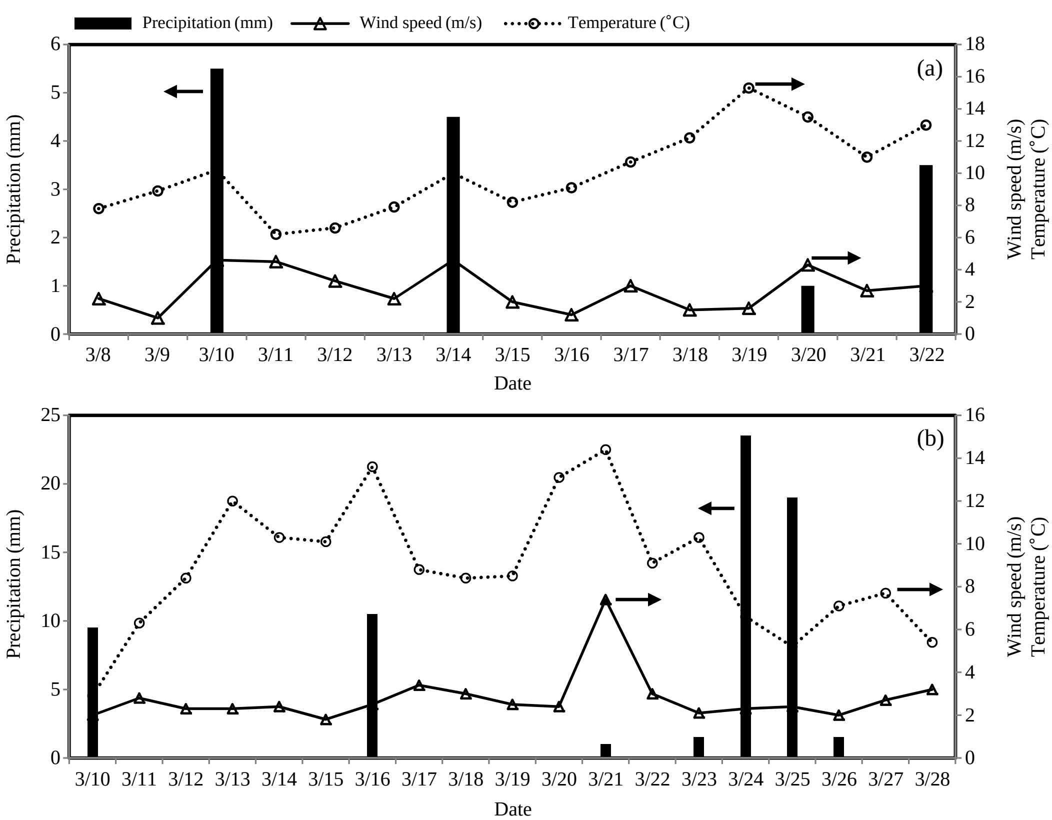

Fig. 4. Daily variation of wind speed, wind direction, temperature and precipitation during the pollen scattering spring seasons in 2009 (a) and 2010 (b).

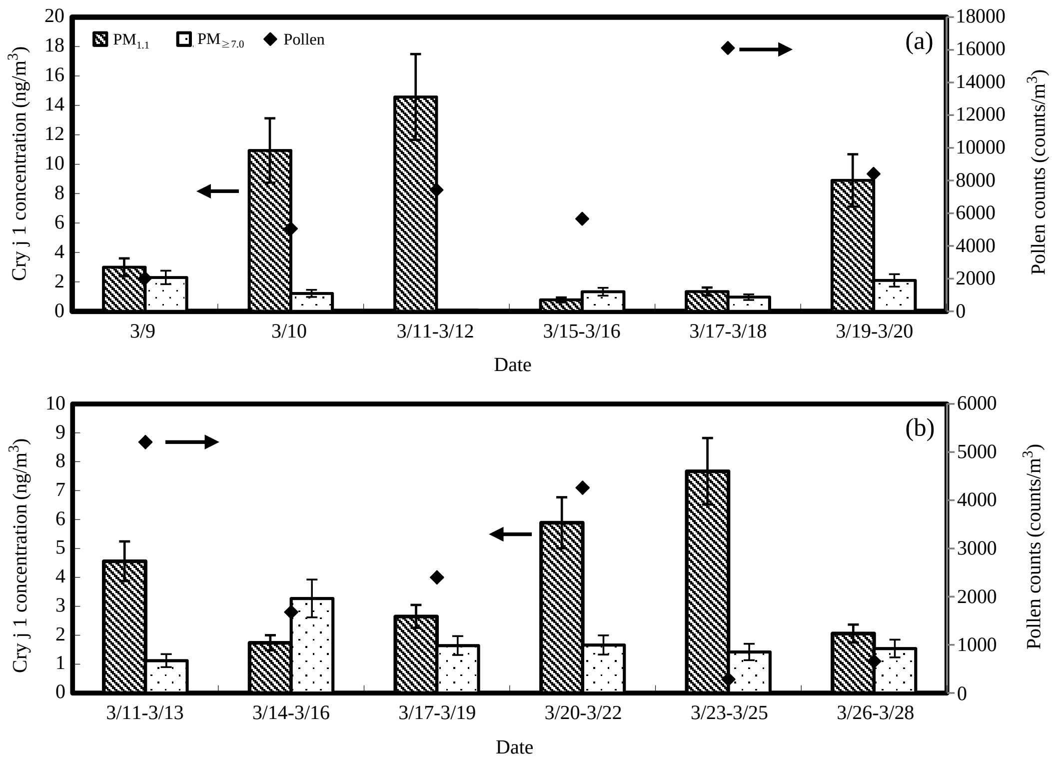

Fig. 5. The concentration of allergen particles Cry j 1 and pollen counts on Mar. 9-20th, 2009 (a) and Mar. 11-28th, 2010 (b).

March 16th (10.5 mm) and 26th (1.5 mm), 2010 (without Yellow Sand events) had rained, Cry j 1 concentration did not increase after rainfall compared with that before rainfall. But on other rainy days Cry j 1 concentrations in $PM_{1.1}$ were almost increased after rainfall. It could be seen that allergenic particles resuspended on sunny days after rainfall.

3. 3 Airborne Behavior of Pollen Allergen during the Yellow Sand Events

In this study, we focused on the airborne allergenic Cry j 1 as the tracer of *Cryptomeria japonica* pollen allergen particles during the Yellow Sand events within pollen scattering seasons in 2009 and 2010. The allergenic Cry j 1 particles in different airborne particles as $PM_{1.1}$ to $PM_{\geq 7.0}$ were collected at the roadside in urban residential zones of Saitama City. Fig. 5 indicated the size-segregated allergenic Cry j 1 concentrations during the Yellow Sand events in 2009 and 2010. From the investigation of the airborne behavior of the size-segregated allergenic Cry j 1 particles, the higher concentrations of the allergenic Cry j 1 were detected in particle size $PM_{1.1}$ than other particle sizes during the Yellow Sand events (March 10-12, 19-20, 2009 and March 20-21, 2010) and after the Yellow Sand events (March 23-25, 2010). However, in 2009, the feature was not identified in March 17-18 during the Yellow Sand events. According to the meteorological data (Fig. 4), we found that on March 10 (5.5 mm) and 20 (1 mm), 2009 and on March 21 (1 mm), 2010 was rainy. Therefore, it was thought that Yellow sand was trapped into rainwater. When Yellow Sand events were transported into Japan, pH and ionic concentrations in rainwater increased drastically (Kawamura *et al.*, 2006). Wang *et al.* (2010) observed that the burst ratio of pollen increased with increasing ionic concentrations. It was also reported that elution of Cry j 1 was induced by solutions with high ionic concentrations (Sagehashi *et al.*, 2005). The concentration of allergenic particles released from pollen was increased (60%) when the sample was in contact with simulated Ca^{2+} containing alkaline rain water (Wang *et al.*, 2012b). Wang *et al.* (2012b) also mentioned that small-sized to fine allergenic particles (as particles below ≤ 1.1 μm, $PM_{1.1}$) created by rainfall containing abundant aqueous inorganic ions. Air polluted rainfall will induce the small-sized *Cryptomeria japonica* pollen allergens.

Table 1 indicates the average pollen counts and average concentrations of Cry j 1 before and after Yellow Sand events. We can see that although the average concentrations of Cry j 1 in $PM_{\geq 7.0}$ were decreased after Yellow Sand events, but the pollen counts and

Table 1. The average pollen counts and average concentrations of allergenic Cry j 1 before and after Yellow Sand events.

Pollen counts and concentrations of allergen		2009		2010	
		Before YS	After YS	Before YS	After YS
Pollen (counts/m^3/24 h)		2000	4700	680	4200
Cry j 1 (ng/m^3/24 h)	$PM_{1.1}$	3.00	4.06	1.24	1.96
	$PM_{\geq 7.0}$	2.30	0.63	0.60	0.55

YS: Yellow Sand event

the average concentration of Cry j 1 in $PM_{1.1}$ were increased (Table 1). Difference between the average concentrations of Cry j 1 in $PM_{1.1}$ before and after Yellow Sand events in 2009 and 2010 were 1.06 ng/m^3/24 h and 0.72 ng/m^3/24 h, respectively. Takahashi *et al*. (2001) reported that symptom of pollinosis appeared when reaching about 1-3 pg/m^3 of *Cryptomeria japonica* pollen allergenic concentrations in the atmosphere. Therefore, worsening asthmatic symptoms may associate with the generation of small-sized allergenic species induced by rainfall and exposed to polluted air, especially Yellow Sand. Difference in the average concentrations of Cry j 1 before and after Yellow Sand events could not be ignored.

Therefore, we suggest that rainfall trapping Yellow Sand is one of the important factors that affect the release of the pollen allergenic species of Cry j 1. Therefore, the further studies on relationships between *Cryptomeria japonica* pollen allergen and Yellow Sand events will be very important.

4. CONCLUSIONS

We investigated the airborne behavior of *Cryptomeria japonica* pollen and its size-segregated allergenic particles during the Yellow Sand events within pollen scattering seasons. From these results, peak of Yellow Sand events and dispersal peaks of pollen grains were observed at same time. Dispersal peaks of airborne pollen grains appeared under higher wind speed and temperature than the previous day. It was thought that Yellow Sand events and airborne pollen counts were related to wind speed. From the investigation of the airborne behavior of the size-segregated allergen particles (Cry j 1), the higher concentrations of the allergenic Cry j 1 in fine particle sizes below 1.1 μm ($PM_{1.1}$) than other particle sizes when the rainwater trapped Yellow Sand. Therefore, we suggest that rainwater trapping Yellow Sand is one of the important factors that affect the release the pollen allergenic specie of Cry j 1. In particular, at the time of Yellow Sand events, we are anxious about aggravation of allergy diseases, such as hay fever and asthma. It had been reported that patients with asthma may thus suffer worsening lower respiratory symptoms due to inflammatory factors in Yellow Sand affecting the nose (Watanabe *et al*., 2011). Yellow Sand may increase airway inflammation, but further studies are needed to define the association between Yellow Sand and the pollinosis. It is also necessary to identify the chemical contents of the Yellow Sand particles that influence *Cryptomeria japonica* pollen allergen. Therefore, the research related with *Cryptomeria japonica* pollen allergen, rainfall and Yellow Sand events, etc. will be important from now on.

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