Japanese Cedar (*Cryptomeria japonica* D. Don) Pollen Information

Subjects: Allergy

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About 40% of cedar pollinosis patients living in the Yamagata Prefecture showed pollinosis symptoms before the first day of the pollen season, which was determined by Durham samplers, the standard sampler for pollen information in Japan. The amount of Cry j 1 (major cedar pollen allergen) per cedar pollen is reported to be six pg. This amount is difficult to measure using the ELISA method. It revealed that Cry j 1 exists in orbicles and tapetum. It is presumed that it is smaller than pollen, so it comes from a place where cedar are already in bloom. It is desirable to obtain real-time information on an hourly basis. Currently, information from automatic cedar pollen monitors is becoming main-stream. However, this monitor may count during snowfalls, Asian dust flying, etc., even when there was no apparent pollen examined with a microscope.

Keywords: Cry j 1 ; ESR (electron spin resonance) radical immunoassay ; Japanese cedar pollen ; monitoring

1. Do Cedar Pollen Allergens Appear before Cedar Pollen in the Air?

Is the amount of cedar pollen allergens always proportional to the numbers of cedar pollen? To clarify this question, the researchers established a method to measure airborne cedar pollen allergens and compared it with daily cedar pollen counts. The existence of micronic allergens in pollen causes many pollinosis, such as Ambrosia [1], Betula [2][3], Gramineae [4], and cedar [5] pollen has been reported. Agarwal et al. [6][7] sampled air samples on fiberglass with a high-volume air sampler and quantified them with the RAST inhibition test. They said particulate aeroallergens may exist in amorphous forms as well as in pollen grains and fungal spores, and symptoms of allergic diseases presumably correlate with the total amount of allergen exposure. The causative agent of cedar pollinosis is cedar pollen allergens. Therefore, a more relevant parameter may be the actual concentration of airborne allergenic particles rather than pollen grains. Since Cry j 1 is the most causative allergen in the pollen, the researchers decided to target this allergen.

In order to answer the question, “Are airborne cedar pollen and airborne cedar pollen allergens always proportional?”, from the analysis using Andersen multi-stage air sampler, Cry j 1 was detected even in small-sized fractions that did not contain cedar pollen, indicating the existence of a pollen-free amorphous form Cry j 1 [8]. Similar results were obtained with birch pollen and birch pollen allergen (Bet v) [9].

Schumacher et al. [10] developed a method for collecting allergens from the pollen of Bermuda grass of Gramineae in the air with a Burkard sampler (seven day recording volumetric spore trap). They transferred them to a nitrocellulose membrane, treated with FITC-labeled antibodies, and observed with a fluorescence microscope. The researchers used their method to compare the number of cedar pollen allergens (Cry j 1) present in the air with that of cedar pollen. The researchers transferred samples to a nitrocellulose membrane in the same way as they did. However, an enzyme-labeled antibody was used for visualization to allow visual confirmation. These Cry j 1-bearing particles can be seen as spots on the membrane [11][12]. **Figure 1** is an example of visualized Cry j 1 spots.

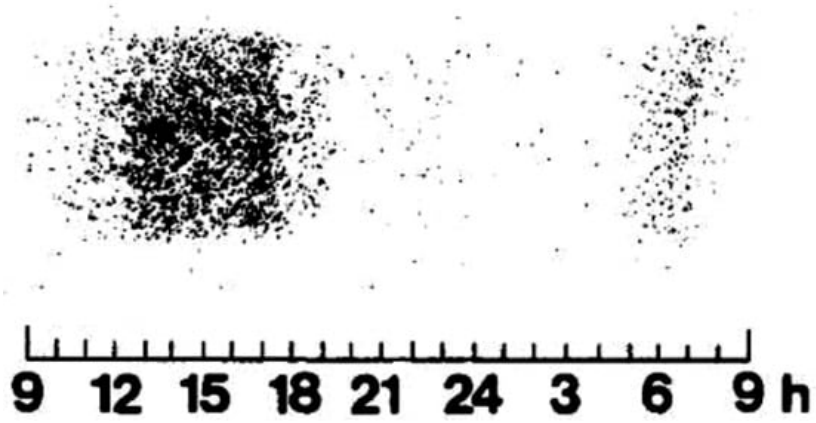

Figure 1. Example of aeroallergen immunoblotting method obtained on 30 March 1991. Cry j 1 spots stained with monoclonal antibody (KW-S91). Visualization was performed with alkaline phosphatase conjugated anti-mouse IgG [11].

The researchers also checked if this immunoblotting technique is applicable to pollen other than cedar. Studies targeting the Lol p allergen in grassland yielded similar results [13] and similar results were also obtained in a study targeting the birch Bet v allergen conducted in Turku, Finland [2]. The researchers also tried using human IgE antibodies from cedar pollinosis patients instead of monoclonal antibodies [14]. This can be applicable not only to pollen, but also to fungi (*Cladosporium*) [15] and mites [16]. The researchers also developed a method to automatically count the number of spots [17]. It became possible to count spots automatically and to obtain the number of spots easily. It is reported that the number of spots is larger than the number of pollen in Bermuda grass [10].

Is there a way to tell which spots are pollen-derived and which are non-pollen spots? Razmovski et al. [18] used samples from the Burkard sampler to allow direct microscopic observation of the particles from which the spots originated. Melinex tape is usually used to collect air samples, but instead of this, acrylic pressure-sensitive adhesive tape (Avery Dennison, Painesville, OH, USA) and PVDF membrane (Amersham Life Science, Hybound type) were used. An antigen–antibody reaction was performed in an aqueous solution while the particles were pressed to identify whether or not the particles possessed the allergen [19]. An example is shown in the illustration of **Figure 2** (cedar pollen) and **Figure 2** (Gramineae pollen). As seen in **Figure 2**, cedar pollen (a–c) has dark spots in the center around the pollen, and thinner spots towards the periphery. Spots with a dark center and a thin periphery can be seen even in particles smaller than pollen (d and e). There were also spots where no particles were found (an example: dotted circle in **Figure 2**a). Similar results were obtained with the grass pollen allergens (**Figure 3**) [19].

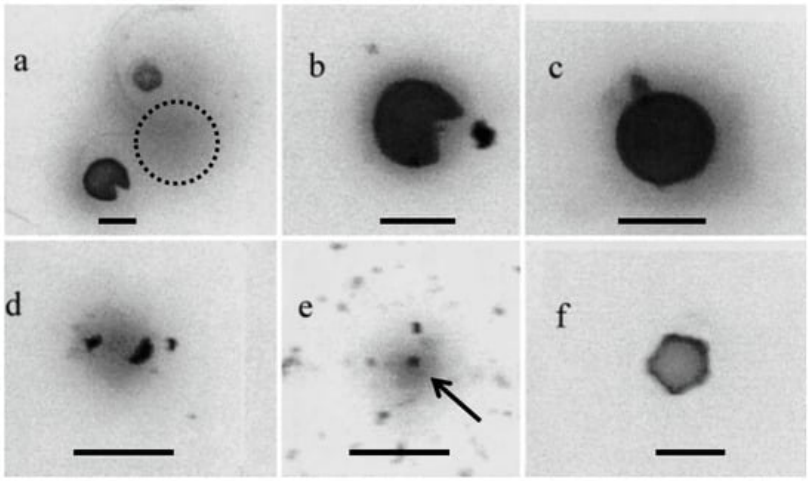

Figure 2. Cry j 1 spots from airborne sample of cedar pollen season treated with anti-Cry j 1 mono- clonal antibody. (**a**–**c**): cedar pollen dotted circle in (**a**): no spot is detected. (**d**,**e**): airborne fine particulate. Arrow indicates orbicles confirmed with an electron microscope, (**f**): *Alnus* spp. pollen. The unit bar in each figure indicates 30 μm. The magnification is different for each figure.

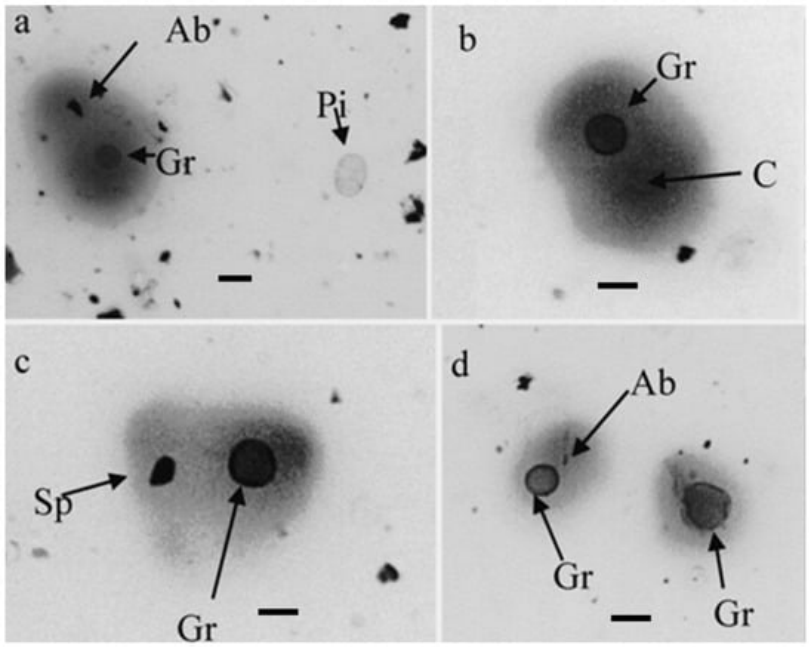

Figure 3. Dac g spots from airborne samples during grass pollen season treated with anti-Dac g rabbit IgG. (**a**–**d**) are areas where spots containing grass pollen were observed. The unit bar in each figure indicates 30 μm. The magnification is different for each figure. Abbreviations are as follows: Ab; airborne fine particulate, Gr; grass pollen, Pi; *Pinus* pollen (no spot is detected), Sp; spore, C; no obvious particle is found.

It was investigated whether this immunoblotting technique could be applicable to pollen allergens other than cedar pollen allergen. Studies targeting the Dac g allergen in the urban area of Yamagata City yielded similar results (**Figure 4**).

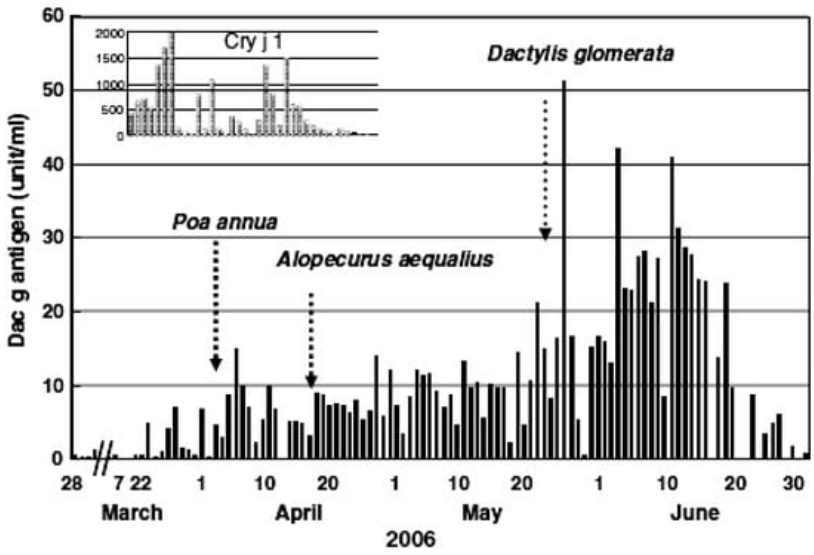

Figure 4. Daily fluctuations of airborne Orchard grass allergen (Dac g allergen) of grass pollen and Cry j 1 allergen measured by immunoblotting in the urban area of Yamagata City where there is no large community [20].

2. Particles Containing Cry j 1 Other Than Cedar Pollen Present in the Air

Then, what kind of cedar pollen allergens containing particles exist in the air before the pollen season? Cedar pollen is differentiated by the summer of the previous year. In order to know at what stage the male flower bud begins to produce cedar pollen allergens, the researchers investigated the Cry j 1 (major cedar pollen allergen) level at each stage of the male flower bud from September of the previous year, the time male flower buds begin to differentiate (pollen tetrad), to March when dispersal begins (mature pollen). As a result, Cry j 1 was not detected at the tetrad stage, and even immature pollen in November was only 1/10 of the amount of Cry j 1 compared to mature pollen during the pollen season [21].

It is known that there are two kinds of allergens (Cry j 1 [22] and Cry j 2 [23]) in cedar pollen. It was revealed that Cry j 1 exists in orbicles on the surface of the sexine of the pollen by immunocytochemical studies. The origin is in the tapetum (the tissue surrounding tissue), where the Cry j 2 is present in starch granules in the cytoplasm [24][25]. Localization of cross-reactive allergens to Cry j 1 in the pollen grains of *Cupressus arizonica* and *Cupressus sempervirens*

(Cupressaceae) has also been investigated, and the cross-reactive allergens were seen in the orbicles and wall [26]. The role of orbicles is also discussed in *Betula* [27].

There are fine particulate allergens (sometimes called subpollen or size-segregated allergenic particles), in addition to pollen, that cause pollinosis. They have been reported of ragweed [1][28], birch [2][3], grass [4] and cedar. They are subpollen particles (SPP) derived from pollen grains; fine particles might generate from wind induced mechanical rupture [29], orbicles simultaneously released from pollen during flowering [24][27], and starch granules released from pollen grains due to rain [30][31]. In the case of birch pollen, Bet v 1 is mainly found in the starch granules and, to a slight extent, in the orbicles and intine from immunocytochemical study [32][33]. Birch pollen grains were shown to germinate on leaves after light rain and release starch granules [34][35][36]. Asthma due to thunderstorms has also been reported [37]. Simultaneously, a rise in the allergen concentration in the air was measured without the presence of pollen grains [30][31]. In the case of Gramineae pollen, spots against Phl p 5 were present in high numbers, whereas the air contained almost no grass pollen grains [3]. Lol P 9, one of the Gramineae pollen allergens, is localized in starch granules [35]. Suphioglu et al. [38] found starch granules with Lol p 9 allergens released from rye grass pollen in the air after rain. In the case of ragweed, pollen grains release subpollen particles (SPP) of respirable size upon hydration [28]. A similar phenomenon has been reported with birch and cypress pollen [34][39]. Pollen rupture is hypothesized to occur due to the allergens in the fine aerosol fraction (<5 μm). In addition, wind induced impaction might also generate SPP [39]. This release of fine particles from pollen due to rain, wind, etc., has been reported for various types of pollen that cause pollinosis. Wang et al. [39] have observed that higher concentrations of the allergenic Cry j 1 were detected in particle size equal to or less than 1.1 μm (PM 1.1) during Yellow Sand events, especially on rainy days, and the size-segregated cedar pollen allergenic particles increase after rainfall in large cities, as it is thought that the cedar pollen allergen attaches to air pollutants (for example, diesel exhaust gas) and floats in the air. They conclude that rainwater trapping Yellow Sand is one of the important factors that affect the release of allergenic pollen species of Cry j 1.

3. Ultrasensitive Measurement Method for Cry j 1 (ESR Radical Immunoassay)

Figure 5 shows the relationship between the numbers of cedar pollen in the air and the amount of Cry j 1 in 2008 (from 3 March to 25 April). Both values did not always match. This is because the Cry j 1 value includes fine particles that contain Cry j 1 in addition to cedar pollen. Correlation analysis from the first day to the end of cedar pollen season is r = 0.543 (moderate correlation, n = 46, $p < 0.1$) in 2008 [40]. The dashed line box indicates the amount of Cry j 1 at the time when sensitive cedar pollinosis patients exhibited symptoms (from 26 January to 28 February), which occurs before the cedar pollen season. Most sensitive cedar pollinosis patients began experiencing symptoms in the period indicated by the dashed square. Many sensitive patients begin to develop symptoms at airborne concentrations of Cry j 1 about 3 to 20 pg/m^3. Conventional ELISA kits had a sensitivity of 150 to 250 pg/mL [22][41]. This amount is difficult to measure with the kits. This method would be difficult even with other pollen, for example birch pollen: 3.2 pg Bet v 1/pollen [42].

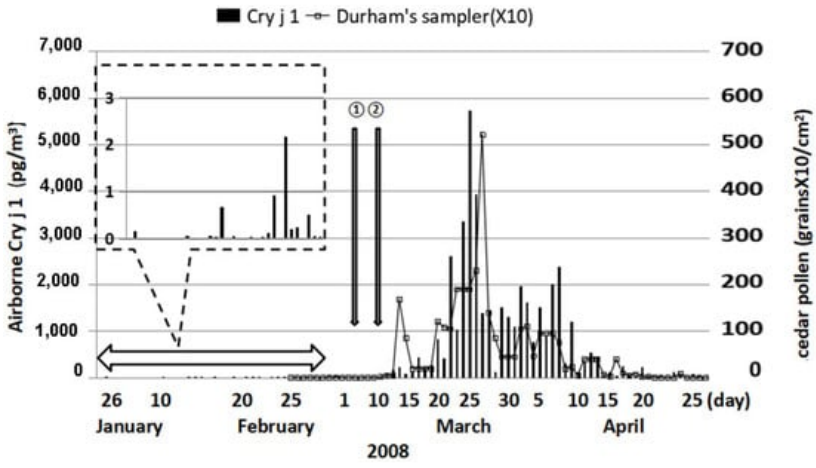

Figure 5. Comparison of daily values of Cry j 1 obtained with ESR radical immunoassay and the cedar pollen with Durham sampler in 2008. The slide glass of the Durham sampler and the sample from cyclone sampler CM90 (Burkard Co., Ltd., Rickmansworth, UK) were exchanged at 7 a.m. every day. The comparison period is cedar pollen season (from the first day of cedar pollen season (11 March) to the end of the pollen season (26 April)). Arrow ①: the first day of cedar pollen observed by microscope, arrow ②: the first day of the cedar pollen season.

ESR radical immunoassay solved this problem [43]. In a broad sense, this assay is an ELISA because it uses an HRP antibody as a secondary antibody. Usually, a chromogenic substrate is used as the enzyme substrate and is quantified

using a spectrometer. Here, a stable radical substrate is used instead of a chromogenic substrate. Radicals are unstable substances, but the reaction product (nitroxide radical) is a stable radical. In this method, an extracted air sample is placed on an anti-Cry j 1 antibody plate (solid phase) and reacted. After the reaction, it is washed and reacted with an HRP (Horseradish peroxidase)—labeled Cry j 1 antibody (secondary antibody). The amount of nitroxide radicals generated as a result of the enzymatic reaction is measured using an ESR (electron spin resonance) device. The amount of Cry j 1 is proportional to the amount of nitroxide radicals. This method was originally developed as an ultra-sensitive measurement method for hepatitis viruses and applied to quantify airborne pollen allergens. Conventional methods require at least 13 pollen grains to be detected, but this method is 100 times more sensitive and can detect even one pollen grain or less. The detection limit was estimated to be 3.5 pg/mL and it is possible to measure 0.1 pg/m^3 of airborne Cry j 1 in a sample which needs 30 µL for each measurement. It became possible to measure a very small amount of Cry j 1 before the first day of cedar pollen season [43]. Rantio-Lehtimaki et al. [2] reported that sensitive birch pollen-allergic patients may experience symptoms when the allergen level reaches about 5 pg/m^3 of Bet v 1. It seems that some patients become symptomatic at roughly the same airborne concentrations of Cry j 1 (cedar pollen) or Bet v 1 (birch pollen). According to a HIALINE study of five European countries, more than 10-fold differences in daily Bet v 1 release were measured, which could be explained by the long range transport of pollen with a deviating Bet v 1 release [44]. Long-distance transportation is possible even in Cry j 1 containing particles. Examining particles emitted from cedar with an Aerosizer, two peaks were obtained. One was the peak of cedar pollen, which had a particle size of 30–37 µm (the average was 34 µm). The other was fine particles of 0.6–1.2 µm (the average was 0.7 µm), and the number of fine particles was more than eight times that of cedar pollen [5]. The fine particulates are considered as ruptured pollen fragments containing the pollen allergens such as tapetum/exime debris and orbicles. They are thought to exist on the surface of pollen grains and allow the pollen grains to exist separately instead of clumping together [24][31]. Since the fine particulates are smaller than pollen, it is thought that they come from a place where cedar is already blooming and cedar pollen cannot be reached [24].

4. Detailed Pollen Monitoring by Real-Time Pollen Monitors

In Japan, pollen other than cedar pollen is rarely seen during the cedar pollen season. Especially during the first half of the dispersal period, most of the airborne pollen is cedar pollen. Even in the latter half of the season, most of it is cedar pollen though there are some areas where cypress pollen is scattered. Therefore, cedar pollen monitors were devised not to identify and count pollen morphology, but instead to identify the size of suspended particles. A laser beam is applied and the intensity of the reflected light is related to size, which is used to determine whether the particle is of interest or not. In order to select spherical particles, beams are irradiated from both vertical and horizontal directions to select pollen grains.

Based on this idea, several types of automatic cedar pollen monitors have been developed **Figure 6** [45][46][47][48]. The first monitors developed (**Figure 6**a,d) had the suction port facing upward. This is because the Durham sampler, which has become a standard sampler, is a sampler that captures naturally falling pollen. These automatic monitors have a good correlation with the Durham sampler and the Burkard sampler, except when it is snowing or when Yellow Sand is coming. Pollen Robo has a suction port at the bottom (**Figure 6**c). **Figure 6**e shows the exterior (outer box) of PS2 pollen sensor by Shin'ei Technology Co. Ltd. Kobe City, Japan. The dashed line box shown on the top left in the figure is the sample suction/measurement part. Because it is attached to the outer box when used, suction can be carried out from various directions. Miki et al. [49] are investigating the effect of orientation of the air inlet and the presence of obstacles near the monitor on airborne pollen concentration. As a result, if the air inlet has a vertical orientation, there is a risk of measurement errors in pollen concentration that are proportional to the vertical wind speed.

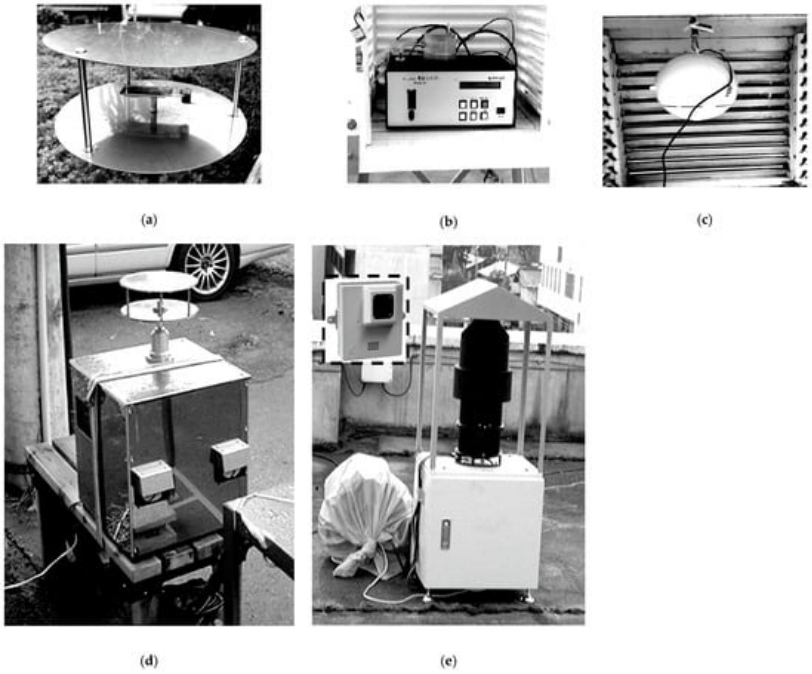

Figure 6. Durham sampler and four types of automatic cedar pollen monitors. (**a**) Durham sampler, (**b**) KH-3000 monitor by Yamato Co., Ltd. Yokosuka City, Japan, (**c**) Pollen Robo by Weathernews Inc. Chiba City, Japan, (**d**) KP-1000 monitor by Kowa Research Institute, Tsukuba City, Japan and (**e**) PS2 pollen senser by Shin'ei Technology Co., Ltd., Kobe City, Japan.

In my experience, the type with the suction port at the bottom is harder to count particles with, other than cedar pollen, but that does not mean it does not count them at all. Then, a type with the suction port at the bottom is preferable since it does not count much during snowfall. The monitor that attracts attention is the "Pollen Robo" developed by Weathernews Inc. One thousand monitors are lent out to volunteers all over Japan every year. Detailed hourly data can be provided in near real-time. "Pollen Robo" is a reliable device during the full-fledged pollen scattering season. The automatic cedar pollen monitor does not look at the cedar pollen itself, and it picks up spherical particles that are the same size as cedar pollen. The monitor developed by the Kowa Research Institute is no different from other monitors in that they measure size, but it also looks at fluorescence, so it does not count particles do not emit fluorescence. In Europe, various types of pollen that cause pollinosis are scattered at the same time, so there is a monitor that can morphologically identify and count each type of pollen [43][50][51]. These automatic pollen monitors look at the morphology of pollen, so they do not count any particles other than pollen. The Pollen Robo is a simple machine that is suitable for detailed data from multiple points, as 1000 monitors are deployed every year.

Is it possible to measure Cry j 1 in a short time, close to real-time if possible? This can be measured using the surface plasmon resonance phenomenon. Because pollinosis is an immediate-type allergic reaction, it develops immediately upon contact with the allergen (within 15 min at the latest). Namely, when pollen comes into contact with the nasal mucosa, allergens are immediately eluted in nasal discharge. Therefore, it is sufficient to extract a sample for a short time from the sample after sampling and send it for measurement. Measurement of eluted allergens with a surface plasmon resonance device yields immediate values [52]. However, the problem is the surface plasmon resonance device is expensive and not suitable for everyday information.

References

1. Agarwal, M.K.; Swanson, M.C.; Reed, D.E.; Yunginger, J.W. Airborne ragweed allergens: Association with various particle sizes and short ragweed plant parts. J. Allergy Clin. Immunol. 1984, 72, 687–693.
2. Rantio-Lehtimäki, A.; Viander, M.; Koivikko, A. Airborne birch pollen antigens in different particle sizes. Clin. Exp. Allergy 1994, 24, 23–28.
3. Yli-Panula, E.; Takahashi, Y.; Rantio-Lehtimäki, A. Comparison of direct immuno staining and electroimmunoassay for analysis of airborne grass-pollen antigens. Allergy 1997, 52, 541–546.
4. Spieksma, F.T.M.; Nikkel, B.; Dijikman, J.H. Seasonal appearance of grass pollen allergen in cultural, micronic aerosol of various size fractions, relationship with airborne grass pollen concentration. Clin. Exp. Allergy 1995, 25, 234–239.

5. Takahashi, Y.; Sasaki, K.; Nakamura, S.; Miki-Hiroshige, H.; Nitta, H. Aerodynamic size distribution of the parti- cles emitted from the flowers of allergologically important plants. Grana 1995, 34, 45–49.
6. Agarwal, M.K.; Yunginger, J.W.; Swanson, M.C.; Reed, C.E. An immunochemical method to measure atmospheric allergens. J. Allergy Clin. Immunol. 1981, 68, 194–200.
7. Agarwal, M.K.; Yunginger, J.W.; Swanson, M.C. Immunochemical quantitation of airborne short ragweed, Alternaria, antigen E, and Alt-I allergens: A two-year prospective study. J. Allergy Clin. Immunol. 1983, 72, 40–45.
8. Takahashi, Y.; Sakaguchi, M.; Inouye, S.; Miyazawa, H.; Imaoka, K.; Katagiri, S. Existense of exine free airborne allergen particles of Japanese cedar (Cryptomeria japonica) pollen. Allergy 1991, 46, 588–593.
9. Takahashi, Y.; Sakaguchi, M.; Von-Pfaler, M.; El-Ghazaly, G. Relationship between numbers of birch pollen and dif- ferent particle sizes of the pollen antigens (Bet v) in the air in Stockholm, Sweden. Allergol. Int. 2003, 52, 111–114.
10. Schumacher, M.J.; Griffith, R.D.; O'Rouke, M.K. Recognition of pollen and other particulate aeroantigens by immunoblot microscopy. J. Allergy Clin. Immunol. 1988, 82, 608–616.
11. Takahashi, Y.; Nagoya, T.; Watanabe, M.; Inouye, S.; Sakaguchi, M.; Katagiri, S. A new method of counting airborne Japanese cedar (Cryptomeria japonica) pollen allergens by immunoblotting. Allergy 1993, 48, 94–98.
12. Takahashi, Y.; Sakaguchi, M.; Inouye, S.; Nagoya, T.; Watanabe, M.; Yasueda, H. Confirmation of the airborne pollen antigen carrying particles by immunoblotting. Allerg. Immunol. 1993, 25, 132–136.
13. Takahashi, Y.; Sakaguchi, M.; Inouye, S.; Yasueda, H.; Shida, T.; Katagiri, S. Airborne grass pollen antigens in a grass- land as studied by immunoblotting with anti-Lol p1 antibody. Grana 1993, 32, 302–307.
14. Takahashi, Y.; Nilsson, S.; Berggren, B. Aeroallergen immunoblotting with human IgE antibody. Grana 1995, 34, 357–360.
15. Suzuki, S.; Takahashi, Y.; Yasueda, H.; Saito, A. Measurement of airborne Cladosporium spp. antigens by aeroallergen immunoblotting and the seasonal fluctuation. Arerugi 2008, 57, 1175–1181.
16. Sakaguchi, M. Measurement of indoor airborne mite allergens. Allergol. Int. 2005, 54, 35–38.
17. Kawashima, S.; Takahashi, Y.; Aikawa, S. An attempt of applying the image processing for the automatic estimation of sampled airborne pollen. Arerugi 1995, 44, 1150–1158.
18. Razmovski, V.O.; Meara, T.S.M.; Tovey, E.R. A new method for simultaneous immunodetection and morphologic identification of individual source of pollen allergens. J. Allergy Clin. Immunol. 2000, 105, 725–731.
19. Takahashi, Y.; Nagoya, T.; Ohta, N. Identification of airborne pollen and airborne particles with pollen allergens (Cry j 1, Dac g) by aeroallergen immunoblotting technique. Arerugi 2002, 51, 609–614.
20. Takahashi, Y.; Aoyama, M.; Abe, E.; Aita, T.; Kawashima, S.; Ohta, N.; Sakaguchi, M. Development of electron spin resonance radical immunoassay for measurement of airborne orchard grass (Dactylis glomerata) pollen antigens. Aerobiologia 2008, 24, 53–59.
21. Takahashi, Y.; Mizoguchi, J.; Katagiri, S.; Sakaguchi, M.; Inouye, S.; Ishikawa, M.; Tonosaki, A.; Iwao, F. Development and distribution of the major pollen allergen (Cry j 1) in the male flower buds of Japanese cedar (Cryptomeria japonica). Arerugi 1989, 38, 1354–1358.
22. Yasueda, H.; Yui, Y.; Shimizu, T.; Shida, T. Isolation and partial characterization of the major allergen from Japanese cedar (Cryptomeria japonica) pollen. J. Allergy Clin. Immunol. 1993, 71, 77–86.
23. Sakaguchi, M.; Inouye, S.; Taniai, M.; Ando, S.; Usui, M.; Matuhasi, T. Identification of the second major allergen of Japanese cedar pollen. Allergy 1994, 45, 309–312.
24. Miki-Hiroshige, H.; Nakamura, S.; Yasueda, H.; Shida, T.; Takahashi, Y. immunochemical localization of the allergenic proteins in the pollen of Cryptomeria japonica. Sex. Plant Reprod. 1994, 7, 95–100.
25. Nakamura, S. Immunocytochemical localization of the allergenic proteins of Japanese cedar pollen and their origin. Kenbikyo (Microscope) 2006, 42, 50–54.
26. Suárez-Cervera, M.; Takahashi, Y.; Vega-Maray, A.; Seoane-Camba, J.A. Immunocytochemical localization of Cry j 1, the major allergen of Cryptomeria japonica (Taxodiaceae), in Cupressus arizonica and Cupressus sempervirens (Cupressaceae) pollen grains. Sex Plant Reprod. 2003, 16, 9–15.
27. El-Ghazaly, G.; Takahashi, Y.; Nilsson, S.; Grafstrom, E.; Berggren, B. Orbicles in Betula pendula and their possible role in allergy. Grana 1996, 34, 300–3004.
28. Smiljanic, D.; Apostolovic, S.; Trifunovic, J.; Ognjenovic, M.L.; Mihajlovic, L.; Burazer, M.; van Hage, T.; Velickovic, C. Subpollen particles are rich carriers of major short ragweed allergens and NADH dehydrogenases: Quantitative proteomic and allergomic study. Clin. Exp. Allergy 2017, 47, 815–828.

29. Visez, N.; Chassard, G.; Azarkan, N.; Naas, O.; Sénéchal, H.; Sutra, J.-P.; Poncet, P.; Choël, M. Wind-induced mechanical rupture of birch pollen: Potential implications for allergen dispersal. J. Aerosol Sci. 2015, 89, 77–84.

30. Schäppi, G.; Taylor, P.E.; Staff, I.A.; Suphioglu, C.; Knox, R.B. Source of Bet v 1 loaded inhalable particles from birch revealed. Sex. Plant Reprod. 1997, 10, 315–323.

31. Schäppi, G.; Taylor, P.; Staff, I.; Rolland, J.; Suphioglu, C. Immunologic significance of respirable atmospheric starch granules containing major birch allergen Bet v 1. Allergy 1999, 54, 478–483.

32. El-Ghazaly, G.; Nakamura, S.; Takahashi, Y.; Cresti, M.; Walles, B.; Milanesi, C. Localization of the major allergen Bet v 1 in Betula pollen using monoclonal antibody labeling. Grana 1996, 35, 369–374.

33. El-Ghazaly, G.; Moate, R.; Cresti, M.; Walles, B.; Takahashi, Y.; Ferreira, F.; Obermeyer, G. Localization and release of antigens from tapetum and pollen grains of Betula pendula. Protoplasma 1999, 208, 37–46.

34. Sénéchal, H.; Visez, N.; Charpin, D.; Shahali, Y.; Peltre, G.; Biolley, J.-P.; Lhuissier, F.; Couderc, R.; Yamada, O.; Malratdomenge, A.; et al. A Review of the effects of major atmospheric pollutants on pollen. grains, pollen content, and allergenicity. Sci. World J. 2015, 2015, 940243.

35. Singh, M.B.; Hough, T.; Theerakulpist, P.; Avjioglu, A.; Davies, S.; Smith, P.M.; Taylor, P.; Simpson, R.J.; Ward, L.D.; McCluskey, J.; et al. Isolation of cDNA encoding a newly identified major allergic protein of rye-grass pollen: Intercellular targeting to amyloplast. Proc. Natl Acad. Sci. USA 1991, 88, 1384–1388.

36. Taylor, P.E.; Flagan, R.C.; Miguel, A.G.; Valenta, R.; Glovsky, M.M. Birch pollen rupture and the release of aerosols of respirable allergens. Clin. Exp. Allergy 2004, 34, 1591–1596.

37. Taylor, P.E.; Jacobson, K.W.; House, J.M.; Glovsky, M.M. Links between pollen, atopy and the asthma epidemic. Int. Arch. Allergy Immunol. 2007, 144, 162–170.

38. Suphioglu, C.; Singh, M.B.; Taylor, P.; Bellomo, R.; Holmes, P.; Puy, R.; Knox, R.B. Mechanism of grass pollen-induced asthma. Lancet 1992, 339, 567–572.

39. Wang, Q.; Gong, X.; Suzuki, M.; Lu, S.; Sekiguchi, K.; Nakajima, D.; Miwa, M. Size-segregated allergenic particles released from airborne Cryptomeria japonica pollen grains during the Yellow Sand events within the pollen scattering seasons. Asian J. Atmos. Environ. 2003, 7, 191–198.

40. Aita, T.; Mogami, K.; Takahashi, Y.; Abe, E.; Aoyama, M. A survey of the Cryptomeria japonica pollen and the allergen of Cryptomeria japonica (Cry j 1) in Yamagata-City. Rep. Yamagata Prefectual Inst. Public Health 2008, 41, 20–22.

41. Watanabe, M.; Tamura, M.; Nagoya, T.; Takahashi, Y.; Katagiri, S. An enzyme-linked immune-sorbent assay for the quantitation of the major allergen from Japanese cedar (Cryptomeria japonica) pollen, Cry j 1, using monoclonal antibody. Arerugi 1992, 41, 1535–1539.

42. Buters, J.T.M.; Antunes, C.; Galveias, A.; Bergmann, K.C.; Thibaudon, M.; Galan, C.; Schmidt-Weber, C.; Oteros, J. Pollen and spore monitoring in the world. Clin. Transl. Allergy 2018, 8, 9.

43. Aoyama, M.; Takahashi, Y. Development of super-sensitive radical immunoassay for Cry j 1. Arerugi 2004, 53, 1089–1090.

44. Buters, J.T.M.; Thibaudon, M.; Smith, M.; Kennedy, R.; Rantio-Lehtimäki, A.; Albertini, R.; Reese, G.; Weber, B.; Galan, C.; Brandao, R.; et al. Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. Atmos. Environ. 2012, 55, 496–505.

45. Kawashima, S.; Clot, B.; Fujita, T.; Takahashi, Y.; Nakamura, K. An algorithm and a device for counting airborne pollen automatically using laser optics. Atmos. Environ. 2007, 41, 7987–7993.

46. Kawashima, S.; Thibaudon, M.; Matsuda, S.; Fujita, T.; Lemonis, N.; Clot, B.; Oliver, G. Automated pollen monitoring system using laser optics for observing seasonal changes in the concentration of total airborne pollen. Aerobiologia 2017, 33, 351–362.

47. Mitsumoto, K.; Yabusaki, K.; Aoyagi, H. Classification of pollen species using autofluorescence image analysis. J. Biosci. Bioeng. 2009, 107, 90–94.

48. Mitsumoto, K.; Yabusaki, K.; Kobayashi, K.; Aoyagi, H. Development of a novel real-time pollen-sorting counter using species-specific pollen autofluorescence. Aerobiologia 2010, 26, 99–111.

49. Miki, K.; Kawashima, S.; Fujita, T.; Nakamura, K.; Clot, B. Effect of micro-scale wind on the measurement of airborne pollen concentrations using volumetric methods on a building rooftop. Atmos. Environ. 2017, 158, 1–10.

50. Oteros, J.; Pusch, G.; Weichenmeier, I.; Heimann, U.; Möller, R.; Röseler, S.; Traidl-Hoffmann, C.; Schmidt-Weber, C.; Buters, J.T.M. Automatic and online pollen mon itoring. Allergy Immunol. 2015, 167, 158–166.

51. Šaulien, I.; Šukien, L.; Daunys, G.; Valiulis, G.; Vaitkevičius, L.; Matavulj, P.; Brdar, S.; Panic, M.; Sikoparija, B.; Clot, B.; et al. Automatic pollen recognition with the Rapid-E particle counter: The first-level procedure, experience and next

steps. Atmos. Meas. Tech. 2018, 12, 3435–3452.

52. Takahashi, Y.; Ohashi, T.; Nagoya, T.; Sakaguchi, M.; Yasueda, H.; Nitta, H. Possibility of real-time measurement of an airborne Cryptomeria japonica pollen allergen based on the principle of the surface plasmon resonance. Aerobiologia 2001, 17, 313–318.