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Original Article

Observations of airborne *Cryptomeria japonica* pollen in the summer

Eri Yoshii,[1] Hideaki Taira,[1] Hidetoyo Teranishi,[2] Yukiko Kenda,[2] Yoko Fujisaki[3] and Maki Saito[4]

[1]Niigata University Graduate School of Science and Technology, [3]Fujisaki Clinic, Niigata, [2]Department of Public Health, Faculty of Medicine, Toyama Medical and Pharmaceutical University and [4]Forest Experiment Station, Toyama Forestry and Forest Products, Research Center, Toyama, Japan

Abstract

Background: Airborne *Cryptomeria japonica* pollen has been detected from July to September, after the main pollen dispersal season (during florescence), in Toyama and Niigata prefectures, Japan.

Methods: To identify the source of the pollen, two possible pathways were investigated: (i) secondary pollen dispersal from the ground; and (ii) release from male flowers remaining on the tree crown in *C. japonica* forests.

Results: Secondary pollen dispersal from the ground ceased just after the main pollen dispersal season ended. However, male flowers remained on the crown until the end of September and released significant amounts of pollen, which contained as much allergenic Cry j 1 as normal pollen dispersed during the spring. There is a significant positive correlation between airborne pollen counts in the spring (February–May) and summer (June–September).

Conclusions: These results indicate that the main sources of airborne pollen grains during the summer are male flowers remaining on the tree crown after their florescence.

Key words: Cry j 1, *Cryptomeria japonica*, pollen dispersal, summer.

Correspondence: E Yoshii, 8050 Ikarashi Ninocho, Niigata-city, Niigata 950-2181, Japan.
Email: f011212d@mail.cc.niigata-u.ac.jp

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Introduction

Since ancient times, *Cryptomeria japonica* D. Don has been one of the most important trees in Japan. *Cryptomeria japonica* trees have straight stems with soft wood that could be easily split, even using ancient implements. Thus, it was frequently used for the construction of houses and many other objects for daily use. The area covered by *C. japonica* forests today has reached 4.5 million hectares, accounting for 45% of the afforested area in Japan.[1] In early spring, *C. japonica* disperses abundant pollen containing allergens, called Cry j 1 and Cry j 2, which induce *C. japonica* pollinosis.[2] The prevalence of this condition has increased yearly and now affects over 10% of the Japanese population.[3] *Cryptomeria japonica* pollinosis has become a serious social health problem.

Male flowers grow at the top of the axis of new needles from July through to August and mature until November. In early September, most of the juvenile pollen grains reach the tetrad stage. They finally reach the mature stage with two nuclei by mid-October or early November.[4–6] Pollen emission begins in the middle of February, when rachises grow, and pollen emissions cease almost entirely in early May.[7] Taira *et al*.[8] have reported that some *C. japonica* trees release pollen from October to January, before the main pollen dispersal season. This pollen is dispersed either via flowering or withering of the male flowers before their recognized flowering season. Therefore, *C. japonica* pollen grains are not expected to occur in the air from June to September. However, the authors of that study detected low levels of airborne *C. japonica* pollen grains from June to September in the period 1988–98 using a daily

airborne pollen survey.[8] The airborne *C. japonica* pollen grains detected from June to September were presumed to have originated either from secondary dispersal of pollen remaining on *C. japonica* trees after their florescence, or from accumulated pollen on the ground. In the present study, these possibilities were examined in order to determine the source of *C. japonica* pollen grains detected during the summer.

METHODS

Airborne pollen counts

The airborne pollen survey was conducted using a Durham sampler.[9] The samplers were set in Toyama (36°40′N, 137°07′E, altitude 90 m) and Niigata prefectures (37°55′N, 139 ÷ 03′E, altitude 20 m) in Japan from 1988 to 1998 (Fig. 1). A glass slide smeared with petrolatum was collected every day and the pollen on the slide was dyed with methyl violet. The number of pollen grains in a 1 cm^2 area was counted under a microscope at ×200 magnification. The daily pollen counts were added together throughout the pollen scattering season and were expressed as yearly pollen counts (count/cm^2 per year).[10]

Survey of male flowers remaining on tree crowns after the main *C. japonica* pollen dispersal season

Study plots were established in three *C. japonica* forests, designated as plot A (33-years-old, altitude 870 m), plot B (25-years-old, altitude 500 m) and plot C (60-years-old, altitude 70 m) in Niigata prefecture (Fig. 1). Between 38 and 66 *C. japonica* trees, each bearing many male flowers, were selected from the forests in these plots.

The male flowers remaining on the crowns were surveyed on 7 May, 5 July, 18 August and 24 September

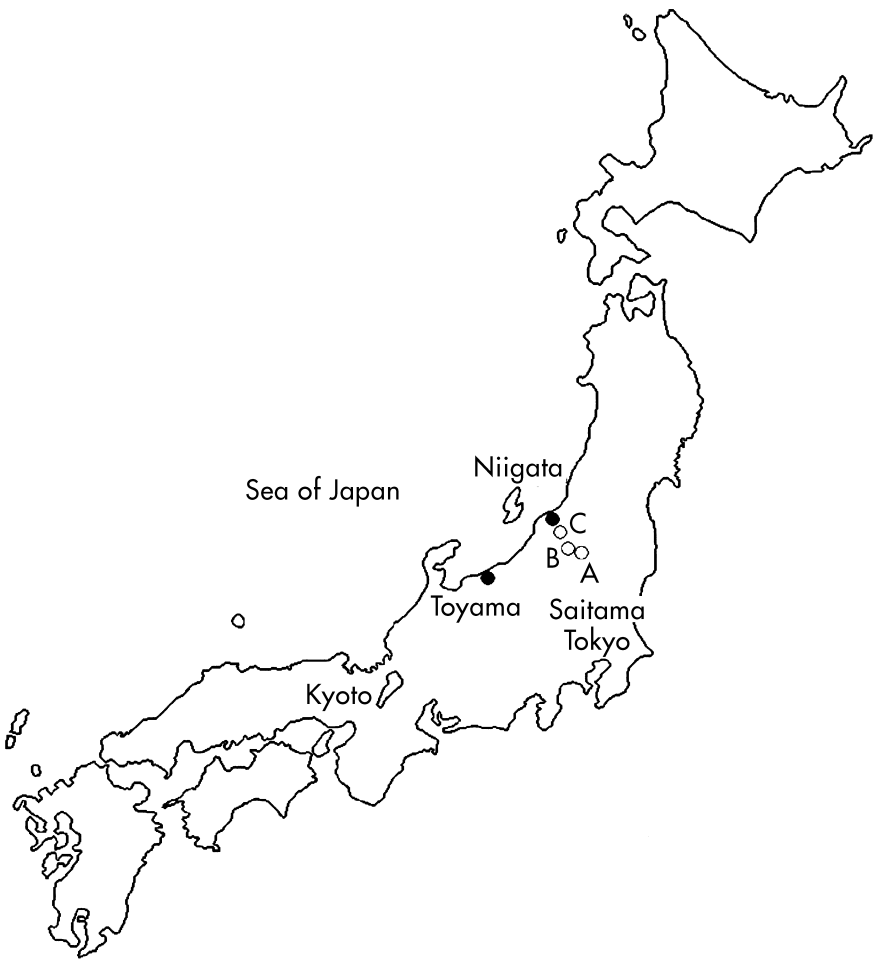

Fig. 1 Positions for the airborne pollen survey (●) and study plots A, B and C (○) in *Cryptomeria japonica* forests.

1988 at plots A, B and C and observations were also made on 10 June at plot A in 1998.

In addition, male flowers remaining on the crown of 66 trees at plot A were observed using a telescope from May to September 1998. The number of male flowers on the crown on 7 May, when male flowers begin to emit pollen, was standardized to 100% for each of these trees. In later observations, the percentage of male flowers remaining on each crown was expressed as a percentage of this figure.

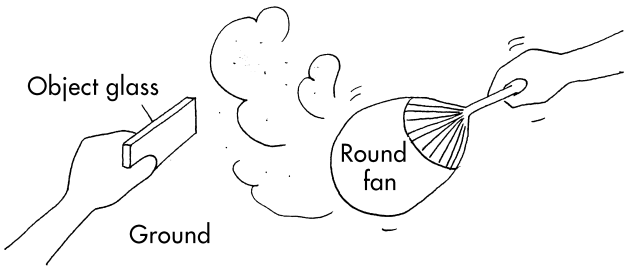

Fig. 2 Survey of secondary pollen dispersal from the ground.

Male flowers collected from *C. japonica* trees were observed under a stereoscopic microscope in August and September, to assess whether or not they contained pollen. To evaluate pollen dispersal from the remaining male flowers on the crown, male flowers were collected on 20 August and 24 September 1998 from 14 and 20 trees, respectively, at plot A, 12 trees at plot B and eight trees at plot C. These male flowers were shaken in a vinyl bag in which a glass slide smeared with petrolatum had been set in the upper part of the bag. Pollen grains on the glass slides were dyed with methyl violet and were counted under a microscope at ×200 magnification. The number of pollen grains found was expressed as counts/1 cm^2 area.

Survey of secondary pollen dispersal from the ground

Secondary *C. japonica* pollen dispersal was examined near *C. japonica* forests from 21 March to 7 May 1995. Surveyed points were located on a tree stump, on litter and on a concrete bridge in the *C. japonica* forests. A glass

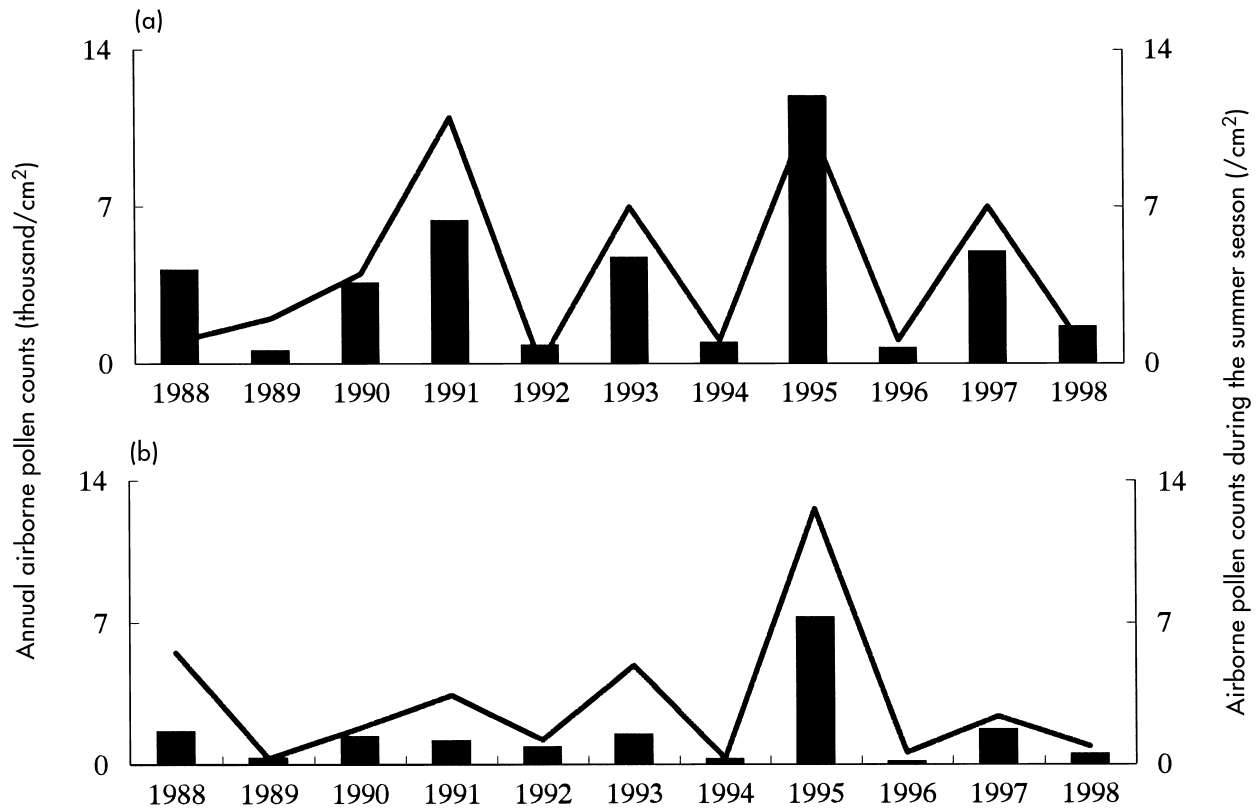

Fig. 3 Annual (■) and summer (June–September; ——) *Cryptomeria japonica* airborne pollen counts in Toyama and Niigata prefectures.

slide, smeared with petrolatum, was set vertically at each point and the ground near the glass was fanned to collect rescattered pollen on one side of the vertical glass slide (Fig. 2). The surveys were repeated five times at each point and mean numbers of pollen grains found were used for analysis.

Measurement of Cry j 1 pollen remaining in male flowers

Male flowers were collected from 13 *C. japonica* trees at plot A on 24 September 1998 and the Cry j 1 content of the pollen remaining in the male flowers was quantitated according to the method of Saito and Teranishi.[11]

Statistical analysis was performed using the SPSS statistical package (SPSS Institute, Tokyo, Japan).

RESULTS

There were large variations in the annual and summer (June to September) airborne *C. japonica* pollen counts between 1988 and 1998 in both Toyama and Niigata prefectures (Fig. 3). There were significant positive correlations between the spring (February–May) and summer

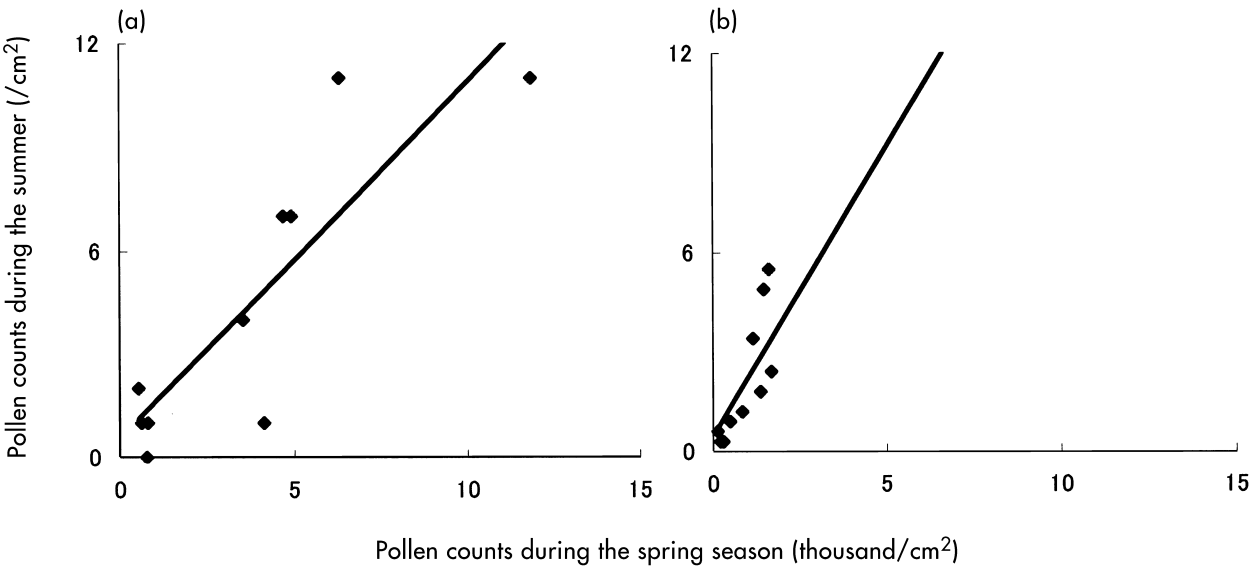

Fig. 4 Correlation between spring (February–May) and summer (June–September) airborne *Cryptomeria japonica* pollen counts in (a) Toyama and (b) Niigata prefectures. For (a): $y = 1.0314x + 0.5625$, $r^2 = 0.7486$; for (b): $y = 0.5177x - 0.0955$, $r^2 = 0.90$.

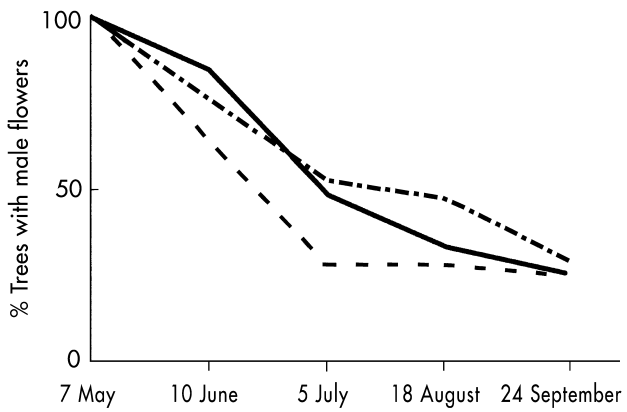

Fig. 5 Percentage of *Cryptomeria japonica* trees having male flowers on the crown at the three plots from 7 May to 24 September 1998. (——), plot A; (-·-·-·-), plot B; (-----), plot C.

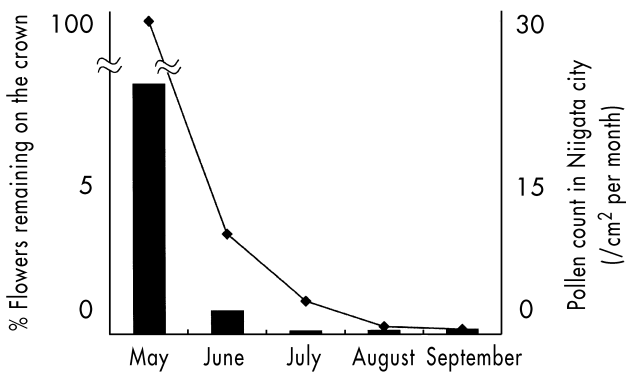

Fig. 6 Variation of the percentage of *Cryptomeria japonica* male flowers at plot A (◆) and the airborne pollen in the urban city (■) from May to September 1998.

(June–September) counts ($R^2 = 0.759$, $P < 0.01$ at Toyama; $R^2 = 0.99$, $P < 0.01$ at Niigata; Fig. 4).

Male flowers began to fall from tree crowns just after flowering. However, in each plot, some male flowers remained on the crown of 20–25% *C. japonica* trees until the middle of September (Fig. 5). Most male flowers fell within 1 month of their flowering. However, approximately 3.2% of male flowers remained on the crown until at least 10 July and 0.16% until September at plot A (Fig. 6). Many configurations of male flowers were observed: some male flowers that had bloomed remained among the needles, while others died together with the needles (Fig. 7). Male flowers that had turned brown and withered, without rachis elongation, were also observed (Figs 8,9). All these types of male flower were found on trees at each plot. The frequency of male flowers that had not bloomed increased as the altitude increased. Pollen grains were found in all of these types of male flowers under a microscope.

Flowers collected from the trees in plots A, B and C in August and September released abundant pollen. The mean number of pollen grains detected in August and September on glass slides placed in the vinyl bags was 109 and 44 /cm^2, respectively, while the maximum numbers of pollen were 500 and 869 /cm^2, respectively.

Cry j 1 was detected in pollen from all male flowers sampled, although the level varied considerably between the samples, ranging from 14.26 to 74.73 ng/grain (mean 39.5 ng/grain). These levels of Cry j 1 are as high as those found in pollen released by normal mechanisms in the spring.

A great deal of secondary pollen dispersal from the ground was observed from 21 March to 7 May 1995. For example, the density of the secondary pollen grains was 1894 /cm^2 at the litter trap on 21 March 1995 and 897.2 /cm^2 at the concrete bridge on 6 April 1995. The highest airborne pollen count in the season was 1659 /cm^2 on 20 March 1995. Thus, the highest secondary pollen count exceeded the maximum airborne pollen count. The highest levels of secondary pollen were detected on fine days just after large amounts of airborne pollen had been recorded. There were few secondary pollen grains recorded just after rainy days. However, the number of secondary pollen grains decreased as airborne pollen counts decreased. On 7 May 1995, when most *C. japonica* trees had stopped releasing pollen, the secondary pollen counts were 0.2 /cm^2 on the stump and the concrete bridge and 1.4 /cm^2 on the litter in the *C. japonica* forest (Fig. 10).

Fig. 7 Remaining *Cryptomeria japonica* male flowers at plot A in September 1998.

Fig. 8 A withered male flower on the crown in September 1998.

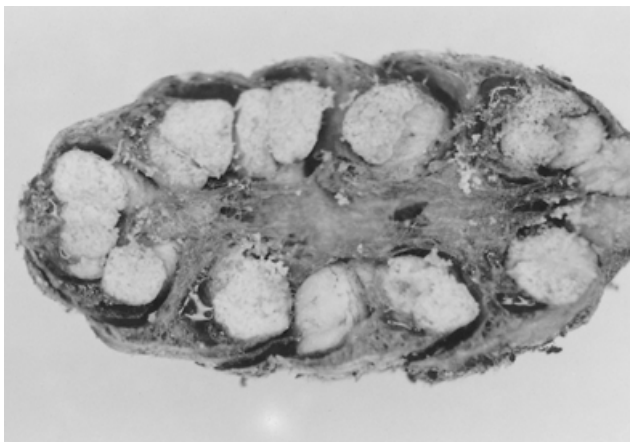

Fig. 9 A withered male flower containing pollen in September 1998.

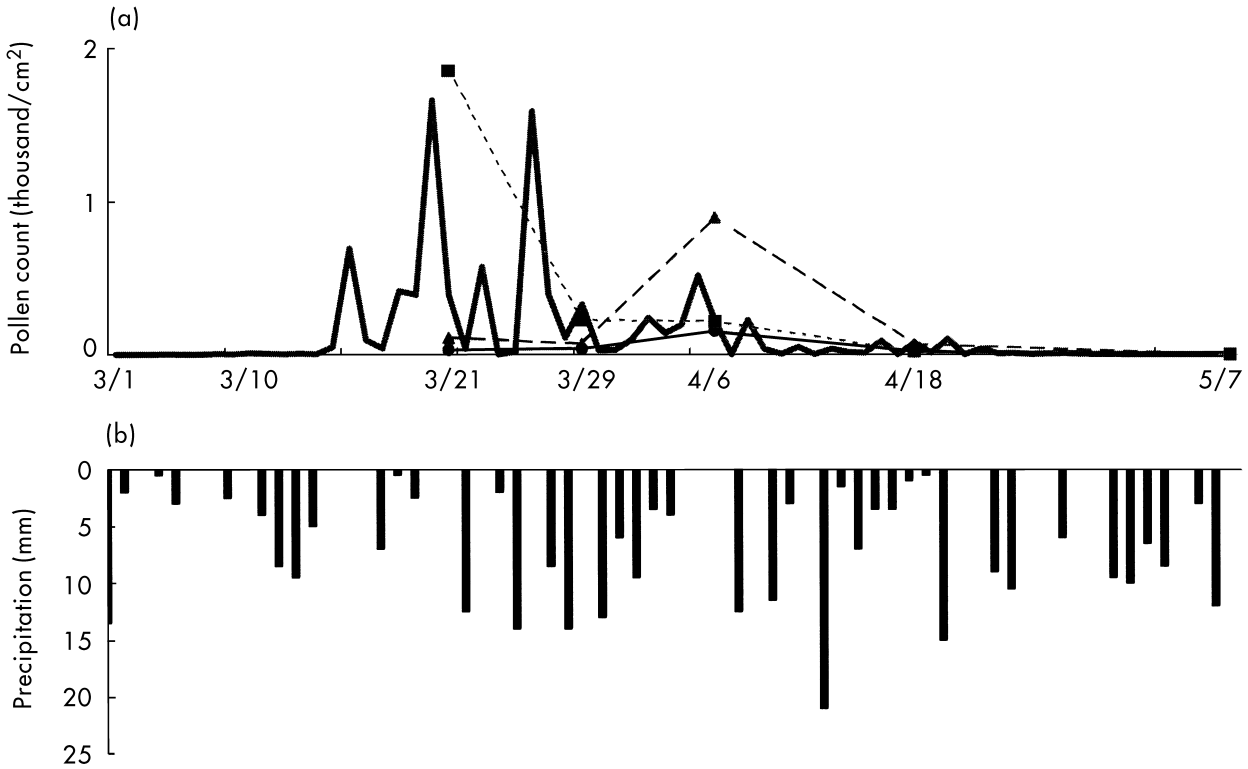

Fig. 10 (a) Variations in airborne *Cryptomeria japonica* pollen counts (———), secondary pollen counts on the tree stump (●), the concrete bridge (▲) and in the litters (■) and (b) precipitation during the pollen dispersal season in 1995.

Discussion

The pollen dispersal season of *C. japonica* ends in early May in both Toyama and Niigata prefectures, but pollen grains were detected from June to September in both regions. Male flowers began to form on *C. japonica* in late June and continued to grow until early September. In early September, most juvenile pollen grains were at the tetrad stage and they finally reached maturity from the middle of October to mid-November. Therefore, *C. japonica* pollen did not enter the air via the usual florescence mechanism during the period from June to September. The source of the airborne pollen between June and September was presumed, instead, to be pollen remaining on *C. japonica* trees or secondary dispersal pollen from the ground.

The abundance of secondary pollen was observed on fine days just after a large amount of airborne pollen had been observed. However, there were very few secondary pollen grains just after rainy days and secondary pollen grains almost disappeared after 7 May.

These results suggest that pollen dispersed from *C. japonica* forests does not accumulate on the ground and that most of the pollen that did fall to the ground was carried away by rainfall to water courses or under the litter, thus preventing it from re-entering the atmosphere.

Sahashi *et al.*[12] have observed airborne *C. japonica* pollen in Tokyo and have suggested that pollen that accumulates on buildings or streets would be rescattered. This could happen in urban areas during the pollen dispersal season. However, there is much precipitation in Japan, especially during the rainy season, in June and July. Annual mean precipitation in Japan generally ranges from 1000 to 2800 mm/year (and may exceed 4000 mm/year in mountainous areas). Thus, most pollen falling to the ground would be either carried away to water courses or trapped under the litter.

Most male flowers fell from tree crowns just after flowering. However, in all *C. japonica* forests studied, 20–25% of trees retained a few male flowers in their crowns until late September. Furthermore, these male flowers released significant amounts of pollen, even in August and September. This evidence strongly suggests that the airborne *C. japonica* pollen found in the summer originates from male flowers retained on the crown.

Keynun *et al*.[13] detected pollen of *Cupressus sempervirens* and *Pinus halepensis* several months after the flowering season. Although they did not discuss the source of the secondary pollen, it was likely to have been generated by the same mechanism as the airborne pollen of *C. japonica* found in the summer. Thus, it is possible that secondary pollen dispersal from retained male flowers could occur from other wind-pollinated species, especially when they bear large numbers of male flowers.

A few *C. japonica* airborne pollen grains were detected during the period from July to September in Saitama prefecture from 1991 to1995.[14] Secondary pollen dispersal from *C. japonica* forests probably occurs during the summer not only in Toyama and Niigata prefectures, but all over Japan, except in Hokkaido and Okinawa, where there are few *C. japonica* plantations.

Cryptomeria japonica pollen counts in the summer were low in the cities of Toyama and Niigata prefectures. However, the secondary pollen contained as much Cry j 1 as the pollen released by the normal mechanism in spring. In regression analysis, *C. japonica* pollen counts during June–September show high positive correlations with pollen counts during February–April. Thus, abundant pollen in the summer season is to be expected following high airborne pollen counts. High levels of airborne pollen should be anticipated near *C. japonica* forests in the summer, especially when large numbers of male flowers have been produced. It is strongly recommended that patients suffering from pollinosis, and physicians, should be made aware of the pollen dispersal that may occur from *C. japonica* forests in the summer.

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