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Original Article

Male flowering index can predict the annual airborne pollen count of *Cryptomeria japonica* at different altitudes

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Abstract

Cryptomeria japonica D. Don (the so-called Sugi or Japanese cedar) is one of the most important coniferous afforestation species. Its afforestation area today has reached 4.5 million hectares, making up 45% of the afforestation area in Japan. *Cryptomeria japonica* pollinosis was first reported in 1964. The prevalence of this disease has increased yearly and now affects over 10% of the Japanease population. In order to establish an accurate prediction method of airborne pollen counts for *C. japonica*, research into the relationship between the amount of airborne pollen, the distribution of *C. japonica* forests and flowering conditions has become very important. In order to clarify differences in airborne pollen counts at different altitudes, four gravity samplers (Durham's type) were set up at four observation points that were located from the coast to the side of a mountain up to 780 m altitude in Toyama Prefecture, Japan. The male flowering index and distribution of *C. japonica* forests were determined in order to evaluate the quantity of male flowers. The relationship between airborne pollen counts, the distribution of *C. japonica* forests and the male flowering index at each observation point was examined. There was a high positive correlation between the male flowering index of *C. japonica* and airborne pollen counts. The male flowering indices, as well as the distribution of *C. japonica* forests at different altitudes, were closely associated with airborne pollen counts at different altitudinal observation points. The flowering index and the distribution of *C. japonica* forests are useful indicators for the accurate prediction airborne pollen counts of *C. japonica*.

Key words: airborne pollen, *Cryptomeria japonica,* male flowering index, method, prediction.

Introduction

An allergy to *Cryptomeria japonica* was first described by Horiguchi and Saito[1] in 1964. Since then, the incidence of Japanese cedar (*C. japonica)* pollinosis has been increasing and its prevalence today has reached over 10% of the total population of Japan.[2,3] Many *C. japonica* trees were planted in forests after World War II. The afforestation area of *C. japonica* has reached 4.5 million hectares and covers 45% of the total afforestation area of Japan.[4] The cutting rotation of *C. japonica* forest was approximately 45 years. It has been reported that the level of pollen production from *C. japonica* forests increases drastically 30 years after plantation.[5] Recently, the afforestation area covered with mature *C. japonica* forests over 45 years of age has been increasing yearly due to social and economic factors. Therefore, the amount of airborne *C. japonica* pollen is predicted to continue to increase over the next several decades.[62]

To prevent symptoms of Japanese cedar pollinosis, it is very important to be able to forecast precisely airborne pollen counts, because the airborne pollen counts directly influence the severity of patients' symptoms[7] as well as levels of IgE antibody.[8] The airborne pollen count is considered to be affected not only by the distribution of *C. japonica* forests in any given area, but also by the

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quantity of male flowers.[9] To predict accurate airborne pollen counts, the relationship between airborne pollen counts, the distribution of *C. japonica* forests and the amount of male flowers must be clarified.[10] Therefore, we studied the significance of the male flowering index of *C. japonica* and airborne pollen counts at different altitudes.

METHODS

Study area

The study area was set up in Toyama Prefecture (36° 17′N to 37°00′ N; 136°46′ E to 137°48′ E), which is located in central Japan and faces the Sea of Japan. To survey airborne pollen counts, four observation points, A (altitude 3 m), B (altitude 230 m), C (altitude 400 m) and D (altitude 780 m) and each in an area with a different distribution of *C. japonica* forests, were set up along the Joganji River, which flows from south to north through the center of Toyama Prefecture (Fig. 1).

Observation point A was located at an altitude of 3 m, 2.8 km south of the seacoast and 10 km from the nearest *C. japonica* forest. Below 50 m altitude, *C. japonica* forests are rare and comprise only 0.6% of the total altitudinal areas of afforestation. Observation point B was located at an altitude of 230 m, 17.0 km south from the coast line and adjacent to a mountainous area in which 30–60-year-old *C. japonica* forests are distributed. The *C. japonica* forests comprise 21% of the total altitudinal area of afforestation at this altitude. Observation point C was located at an altitude of 400 m, 21 km south of the coast line and contained the most abundant distribution of *C. japonica* forests with 60–400-year-old trees. At this altitude, the area covered by *C. japonica* forests comprises 20.1% of the total altitudinal area of afforestation. Observation point D was located at an altitude of 780 m and 25 km south from the coast line. At this altitude, *C. japonica* forests comprise 15.4% of the total altitudinal areas of afforestation.

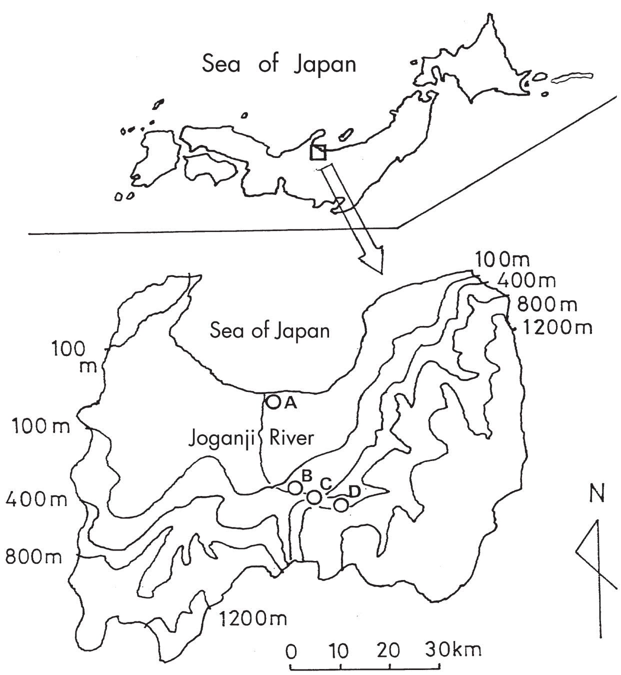

Fig. 1 Study area. The study area was set up in Toyama Prefecture, which is in central Japan and borders the Sea of Japan. Four observation points, namely A (altitude 3 m), B (altitude 230 m), C (altitude 400 m) and D (altitude 780 m), were established along the Joganji River, which flows from south to north through the center of Toyama Prefecture, Japan.

Flowering index

The male flowering index was determined according to the method described previously.[10] Because male flowers grow at the tip of the axil of new needles in July and August and mature until November, the condition of the male flowers on the surface of the crown can be observed after November. At the end of November, 20–30 trees over 30 years of age standing outside *C. japonica* forests were chosen to evaluate the flowering index. The productivity of male flowers in the upper and lower parts of each tree was assessed, because the productivity of male flowers at different crown heights differs.[10] The condition of the male flower on each crown was assigned a score between 0 and 3 as follows: (i) a score of 0 was given if male flowers could not be found on the crown; (ii) if male flowers were observed sporadically on the crown, that flowering condition was scored as 1; (iii) if male flowers were observed over the entire crown surface but were few in number and if male flowers were observed among the needles the flowering score was 2; and (iv) if the tree crown was covered with many male flowers making the needles hard to see, the flowering score was 3. The flowering scores of the upper and lower parts of the tree were gathered. Therefore, the range of flowering scores for a tree ranged from 0 to 6. The mean flowering index was determined as the average scores of 20–30 trees that were over 30 years of age standing outside the *C. japonica* forest that were the closest to each observation point.

Airborne pollen survey

From 1991 to 1996, airborne pollen surveys were conducted using Durham's sampler.[11] Pollen was collected on applied petrolatum glass slides in the sampler every day between February and April. Glass slides were stained with methyl violet and the amount of pollen in 1 cm^2 was counted under a microscope[12] at $\times 200$ magnification. The daily pollen counts were summed throughout the pollen season and were expressed as yearly pollen counts (count/cm^2 per year). Meteorological data were supplied by the Japan Weather Association. The percentage of afforestation areas covered by *C. japonica* forests over 30 years of age at each altitude was calculated from the Basic Forest Census.

Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Tokyo, Japan). The strength of a linear association between flowering index and *C. japonica* pollen counts was measured by the Spearman rank correlation coefficient test.

Results

The mean male flowering index of *C. japonica* at each observation point varied widely by year and by altitude. The higher the airborne pollen counts, the higher the mean male flowering index.

The airborne pollen counts varied widely at the four observation points from 1991 to 1996. They also varied widely in different years at each observation point. The mean total airborne pollen counts of the four observation points was 8136 count/cm^2 per year in the abundant harvest year of 1995 and was 340 count/cm^2 per year in the poor harvest year of 1996. The mean total airborne pollen count in 1995 was 24-fold that in 1996 (Fig. 2).

Three types of relationship between airborne pollen count and male flowering index were noted. The first was seen in abundant harvest years in 1991 and 1995. The total airborne pollen counts were 17 417 and 32 544 count/cm^2 per year in 1991 and 1995, respectively. In 1995, at the four observation points, the highest airborne pollen count was five-fold that of the lowest one. Generally, observation points located in areas with a higher distribution of *C. japonica* forests showed higher airborne pollen counts than those located in areas with a lower distribution of *C. japonica* forest. In 1995, there was little difference in the male flowering index at different altitudes and the male flowering index was generally high.

The second type of relationship was seen in poor harvest years in 1992 and 1996. The total airborne pollen count was 1746 and 1360 count/cm^2 per year in 1992 and 1996, respectively. The airborne pollen counts at the four observation points were nearly the same, even though the afforestation area covered by *C. japonica* forests differed at each observation point. The male flowering index differed according to altitude. The male flowering index was relatively high at lower altitudes and decreased at higher altitudes.

The third type of relationship was intermediate between the abundant and the poor harvest years. The total airborne pollen count was 10 338 and 3963 count/cm^2 per year in 1993 and 1994, respectively. The pollen count differed at the four observation points according to the distribution of *C. japonica* forests around the

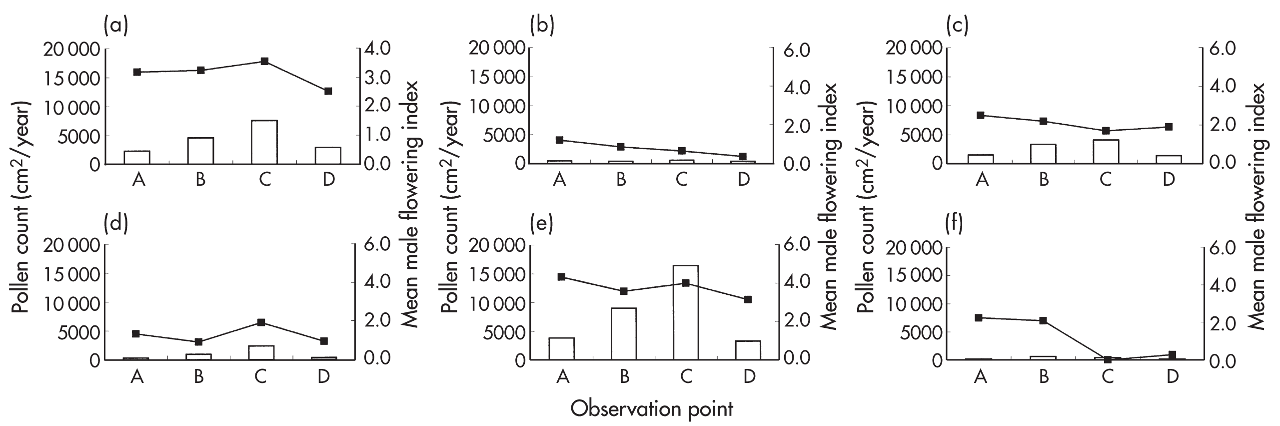

Fig. 2 Total airborne *Cryptomeria japonica* pollen counts (□) and mean male flowering index (–■–) at observation points A–D in (a) 1991, (b) 1992, (c) 1993, (d) 1994, (e) 1995 and (f) 1996. The abundant harvest years were 1991 and 1995 and poor harvest years were 1992 and 1996. Both 1993 and 1994 were deemed as intermediate harvest years.

observation point. The mean male flowering indices in 1993 and 1994 were lower than in 1991 and 1995 and they differed slightly among the four altitudes.

At each observation point, the mean male flowering index near the observation point and the pollen count at the observation point showed a high positive correlation (Fig. 3). In the Spearman rank correlation coefficient test, the male flowering index showed a high positive correlation with the airborne pollen count. The correlation of observation point B was significant at the 0.05 level and that of observation points C and D was significant at the 0.01 level, although there was no significant level at observation point A (Table 1).

Discussion

The study area is located in the center of the main island and faces the Sea of Japan. It is surrounded by high mountains of the Japan Alps, where there are no *C. japonica* trees.[13] Airborne pollen from neighboring areas would not influence the counts in this region, except at the western border. The *C. japonica* trees disperse pollen for the most part during March and April. The weather conditions at the Toyama meteorological observatory show that there is little difference in total precipitation recorded in March and April. In addition, the mean wind velocity recorded in March and April is almost the same and the frequency of the main direction of the wind each day shows almost the same pattern. The main wind direction is dominated by the south-west and north-east. Pollen counts are influenced by weather conditions,[14,15] especially wind velocity and precipitation. In the present study, there was no significant relationship between total airborne pollen count and either precipitation and wind speed in March and April, because there are few differences in precipitation and wind speed among the observed years. The duration of pollen dispersal from *C. japonica* forests lasts for 2 months. The weather conditions change periodically from fine weather to rainy weather during the pollen dispersal season, therefore, the effect of the weather conditions on total

Table 1 Correlation between *Cryptomeria japonica* male flowering index and airborne pollen counts at each observation point

Observation point	Correlation coefficient	Significance (two-tailed)
A	0.771	0.589
B	0.886*	0.190
C	0.943**	0.005
D	0.986**	0.000

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

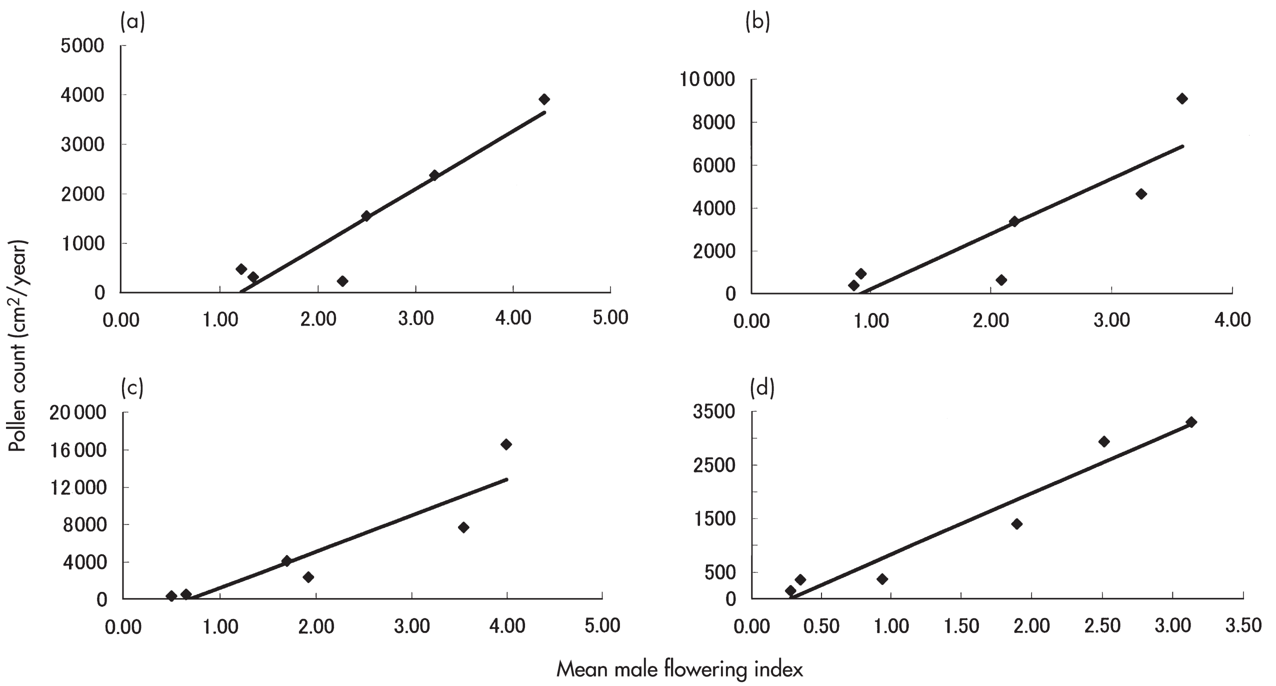

Fig. 3 Relationship between total airborne *Cryptomeria japonica* pollen count and mean male flowering index at observation points A (a), B (b), C (c) and D (d).

airborne pollen counts at the observation points is small. The production of male flowers will mainly affect total airborne pollen counts.

Airborne pollen observation points were established along the Joganji River, which is located in the center of Toyama Prefecture. In the abundant harvest years, airborne *C. japonica* pollen counts at observation points A, B, C and D varied according to the percentage of area covered by *C. japonica* forest. Observation point A, which is 10 km from a *C. japonica* forest, showed the lowest pollen counts. Pollen counts at observation point B were intermediate between those at observation points A and C. Observation point C, which is in the center of an area of abundant *C. japonica* forest, recorded the highest airborne pollen counts.

The airborne pollen counts at observation point D were similar to those at observation point A, although the distribution of *C. japonica* forest near observation point D is higher than that near observation point A. William *et al*.[16] reported that final pollen counts can be attributed to a number of factors. In the study area, when the south wind blew during the pollen dispersal season, the temperature rose and abundant pollen was scattered from *C. japonica* forests towards the north. Observation point D, which is at the southern end of *C. japonica* forest in the region, recorded the lowest airborne pollen counts, although a high percentage of the afforestation area is covered by *C. japonica* forest (Figs 1,4). The male flowering index was not affected by altitude in the abundant harvest years and the flowering index at each observation point was high. This means that abundant pollen was produced in areas with a high density of *C. japonica* forests. For this reason, the variations in airborne pollen counts among the observation points were larger in abundant harvest years. However, in poor harvest years, the male flowering index was lower at higher altitudes. This means that areas of lower altitude produced a comparatively larger amount of pollen and that a small amount of pollen was produced at higher altitudes. Therefore, there was no variation in airborne pollen counts among the observation points in poor harvest years. These phenomena explain why observation point A showed little variation in the amount of airborne pollen and observation point C showed high variation in airborne pollen counts.

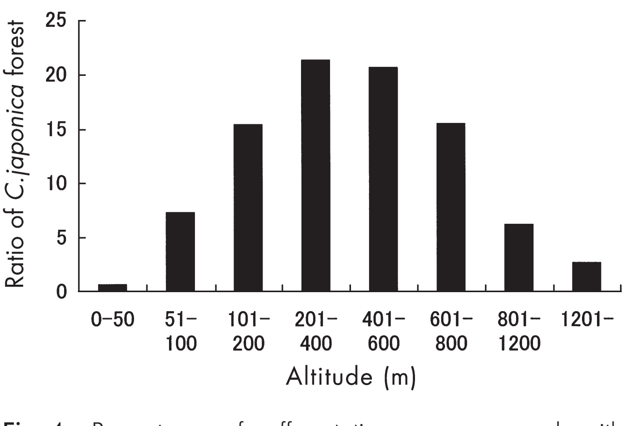

Fig. 4 Percentage of afforestation area covered with *Cryptomeria japonica* forest of the total afforestation area at each altitude range in Toyama Prefecture.

There are large variations in the male flowering index at high elevations. A low male flowering index was recorded in the poor harvest years. Differentiation of male flowers is promoted by high temperatures in July.[17] Because temperature decreases by 0.6°C with each 100 m increase in altitude, the temperature at observation point D is 4.4°C lower than that at observation point A. Consequently, the mean July temperature at high elevations is not enough to promote differentiation of male flowers in the poor harvest years. This may be the reason for a lower flowering index at higher altitudes in the poor harvest years.

In the present study, there was a high positive correlation between the flowering index near each observation point and the airborne pollen count at that observation point. The airborne pollen observation points that are located in areas with a higher distribution of *C. japonica* forests showed higher airborne pollen counts than those located in areas with a lower distribution of *C. japonica* forests. This indicates that the amount of airborne pollen at each observation point was directly affected by male flower production in the nearest *C. japonica* forest. Accordingly, airborne pollen counts at the observation point can be predicted accurately by the distribution of *C. japonica* forest and the male flowering index at the nearest *C. japonica* forest.

In the present study, we used Durham's pollen sampler to determine airborne *C. japonica* pollen. Airborne *C. japonica* pollen counts detected by Durham's sampler showed clear relationships with the male flowering index as well as the distribution of *C. japonica* forests around each observation point. Therefore, the results of the present study indicate that Durham's sampler is a useful tool to survey airborne *C. japonica* pollen counts. Two kinds of samplers are used to determine airborne pollen counts in Japan. One is Durham's pollen sampler, which is a pollen collection device based on the gravity method.

The other is the Burkard Seven-Day Recording Volumetric Spore Trap (Burkard sampler), which determines the amount of pollen in a specific volume of air. Durham's sampler collects lower quantities of pollen than the Burkard sampler.[18,19] Therefore, it may be not suitable for detecting small amounts of pollen in the air. However, Durham's sampler is advantageous in its easy handing, low price and no need for electricity. Consequently, it works in any location. As shown in the present study, Durham's sampler seems to reflect the actual airborne pollen counts of *C. japonica*. This may be explained by the fact that *C. japonica* forests produce a large amount of pollen that is carried some distance by the wind.

Several studies have been performed to try to predict the total airborne pollen counts of the following year.[20–23] These predicting methods commonly use the mean July temperature. However, there are several differences between predictions and actual total pollen counts, especially for few and abundant harvest years.[17] Because the florescence and production of male flowers is affected not only by temperature, physiologic alterations of the trees are important for such predictions.[24] As mentioned above, we demonstrated that flowering indices and distribution of *C. japonica* forests are useful indicators for the prediction of accurate airborne pollen counts. Keynan *et al*.[25] reported a method for forecasting pollen pollution with floral development in Israel, which is characterized by variable climates. Japan is a mountainous and topographically complex country. Vegetation flora changes along with geographic variation and altitude. Consequently, the prediction of *C. japonica* airborne pollen counts by direct observation of male flowers will become an important methodology to establish prevention measures against Sugi pollinosis.

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