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Original Article

The formation of pollen in male flowers and yearly atmospheric pollen counts of *Cryptomeria japonica* in the following year

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Abstract

Prediction of yearly atmospheric pollen counts is a very important component in the prevention of allergenic symptoms. We investigated the relationship between atomspheric pollen counts and the formation of male flowers of *Cryptomeria japonica* D. Don (*C. japonica*). An atmospheric pollen survey of *C. japonica* was conducted from 1983 to 1996 using a Durham's sampler. A regression analysis was performed between the total pollen count and July temperature in previous years. The atmospheric pollen counts of *C. japonica* had a high positive correlation with the mean temperature in July of the previous year. However, the predicted using average mean July temperature records of the previous year were insufficient, especially in years following high pollen count. In experimental conditions, using 60 *C. japonica* trees in pots, the formation of male flowers was shown to increase with a rise in incubation temperature. In a forest of *C. japonica*, our results showed that the length and weight of new needle growth from old needles, which produced many flowers in the previous year, were shorter and lighter, respectively. These aerobiological and plant physiological studies provide evidence that a smaller number of pollen counts are a common result in a year following one in which many male flowers are produced, even if the mean July temperature of that year was high.

Key words: atmospheric pollen, *Cryptomeria japonica*, male flower production, pollinosis.

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Introduction

Cryptomeria japonica D. Don is one of the most important afforestation species in Japan. It grows more rapidly than other native conifers and has straight stems. Thus, *C. japonica* is a very useful timber for house construction in Japan. Forty-five percent of the afforestation area in Japan is covered with *C. japonica*, a total of 4.5 million hectares.[1] The pollen of *C. japonica*, however, is a major causative allergen in Japan.[2,3]

Horiguchi and Saito first described the allergy to *C. japonica* pollen in 1962.[4] It has since increased rapidly[5,6] and now affects approximately 10% of the Japanese population.[7] Therefore, the prevention of allergies to *C. japonica* is an important issue in the country. The precise forecast of atmospheric pollen is useful information for reducing the uncomfortable allergy symptoms of sufferers. As the yearly atmospheric pollen count of *C. japonica* is closely related to the mean temperature in July of the previous year,[8,9] the annual atmospheric pollen count of *C. japonica* can be predicted by a formula which uses the mean July temperature of the previous year. However, the atmospheric pollen counts predicted using the formula are often less in number than the actual atmospheric pollen counts, especially in the years following high levels of flower production. It should be considered that the differentiation of male flowers is not only influenced by climatic and other environmental factors, but also by physiological changes of *C. japonica* itself.

We investigated the atmospheric pollen count of *C. japonica* in Toyama prefecture during the period of 1983–96, studying the relationship between atmospheric pollen counts and climatic factors. We also studied the productivity of male flowers under varied temperature conditions and the alteration of male flower productivity when many male flowers were produced.

Materials and methods

Atmospheric pollen survey

An atmospheric pollen survey was conducted using a Durham sampler.[10] The sampler was set in Toyama, Japan (36°40′N, 137°07′E) from 1983 to 1996. Applied petrolatum slide glasses were collected each day and the pollens were dyed with methyl violet.[11] The pollens on the slide were counted under a microscope of 200 magnification and the sum of the pollen count in each season was expressed as count/cm^2/year.

Experimental studies of the effect of temperature conditions on the differentiation of male flowers

In order to study the temperature conditions from July to September, which influence the amount of male-flower production on *C. japonica*, 60 young trees (mean height 36.9 ± 3.0 cm, mean basal diameter 0.8 ± 0.1 cm) planted in pots were treated with 100 p.p.m. of the growth hormone Gibberellin (Kyowa Corp., Tokyo, Japan). This was to promote the differentiation of male flowering at the end of June 1990. These 60 trees were divided into two groups, the first consisting of 20 trees and the second of 40 trees. Five trees from the first group were incubated for 60 days at 24°C, 26°C, 28°C and 30°C respectively, using incubators (LPH-200 RDSCD; Nihon Ikakikai Corp., Tokyo, Japan. The second group were incubated at 30°C and five of them were transferred to the incubator at 20°C every three days. The luminous intensity of the incubators was set at 20 000 lux for 12 h per day. The numbers of male flowers produced on the trees were counted at the end of October 1990.

Comparison of the growth of new needles

Ten *C. japonica* trees were chosen at random from a 30-year-old forest in the Toyama prefecture. These trees were measured for new needle growth during the period of April to October 1990. In each tree, increment of 40 new needles, those which developed from the old needles were measured every 10 days. Although 20 of the old needles produced many male flowers in the previous year, the remaining old needles did not produce any.

In 1991, six trees (mean diameter 11.9 cm, mean height 7.6 m) were chosen from a 12-year-old forest. Three of these trees produced many male flowers while the others did not produce any male flowers in the previous year. The tree crowns were generally divided into three parts: upper, middle and lower. This was because the productivity of the male flowers differed between the crown heights. Three branches were collected randomly from each part and were divided into four parts: twigs, new needles, old needles and corns. They were dried at 105°C and weighed in October 1991. The data, which consisted of percentages, were transformed into arcsine percentages. For statistic evaluation, both regression analysis and analysis of variance (ANOVA) were used.

Results

Atmospheric pollen survey

The fluctuations of atmospheric *C. japonica* pollen counts from 1983 to 1996 are shown in Fig. 1. The most abundant atmospheric pollen counts were observed in 1995 and the least in 1989. *C. japonica* pollen counts ranged between 500 and 15 000 from 1983 to 1996. There was an indication that the pollen counts were increasing yearly, although the pollen counts varied widely. We decided to divide the pollen counts into three groups: less, intermediate and most abundant. A year in which the pollen count measured less than 1000 was considered a bad harvest year. When more than 4000 were measured, it was considered an abundant harvest year. An abundant harvest appeared irregularly every 2 or 3 years. We analyzed the relationship between atmospheric pollen counts and the weather conditions of the previous year, especially during the differentiation period of male flowers, which is from June to August.[12] In a regression analysis, a pollen count of *C. japonica* showed the highest positive correlation (r = 0.76; P < 0.01) with the mean July temperature of the previous year (Fig. 2).

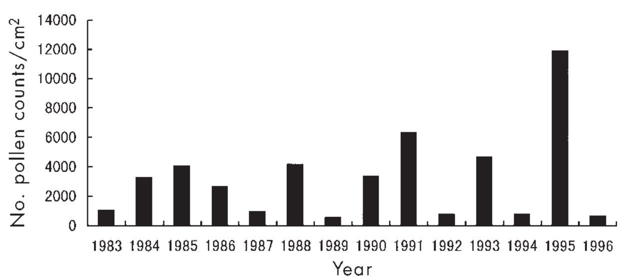

Fig. 1 Atmospheric *C. japonica* pollen counts at Toyama Medical and Pharmaceutical University (36°40′N, 137°07′E).

Effect of temperature conditions on the differentiation of male flowers

C. japonica incubated at 24°C, 26°C, 28°C and 30°C showed large differences in male flower differentiation. No male flowers were found when *C. japonica* was incubated at 24°C, although male flower production increased according to rising incubated temperatures (Fig. 3). In the transfer experiment, the number of the male flowers increased according to the length of the 30°C incubation period (Fig. 4).

Measurements of the growth of new needles

Elongation of the new needles began at the end of April and ceased by the end of October. The needles grew more rapidly from May to June, and gradually slowed down from July to August. The monthly mean elongation of needles was largest in May and decreased from June to August. The mean elongation of new needles that had been developed from old needles that produced male flowers in the previous year was 4.0 cm. The mean elongation of new needles that had been developed from old needles that did not produce any male flowers was 8.9 cm from April to the end of October (Fig. 5). Thus, there was a significant difference between them ($P < 0.01$). In the three *C. japonica* trees that produced a large number of male flowers in the previous year, only 13.1% of the old needles developed new needles. However, in the other three *C. japonica* trees that did not produce any male flowers in the previous year, 42.2% of old needles developed new needles. The ratio of weight increment of new needles (dry weight of new needles/dry weight of old needles $\times$ 100) was 4.8% in the *C. japonica* trees which produced many male flowers in the previous year, while it was 28.6% in the *C. japonica* trees which did not produce any male flowers in the previous

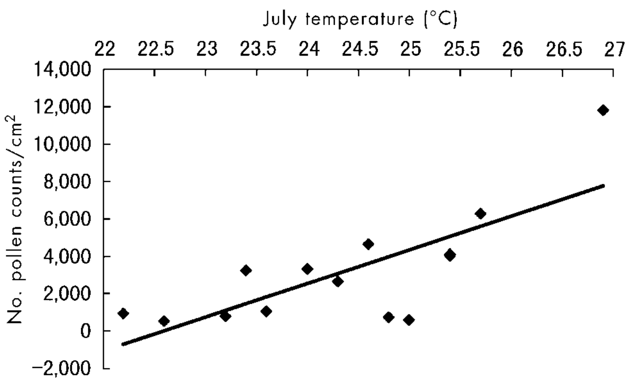

Fig. 2 The correlation between the mean July temperature of the previous year and atmospheric pollen counts of *C. japonica* from 1983 to 1996. $y = 1814.8x - 41010$; $r = 0.76$.

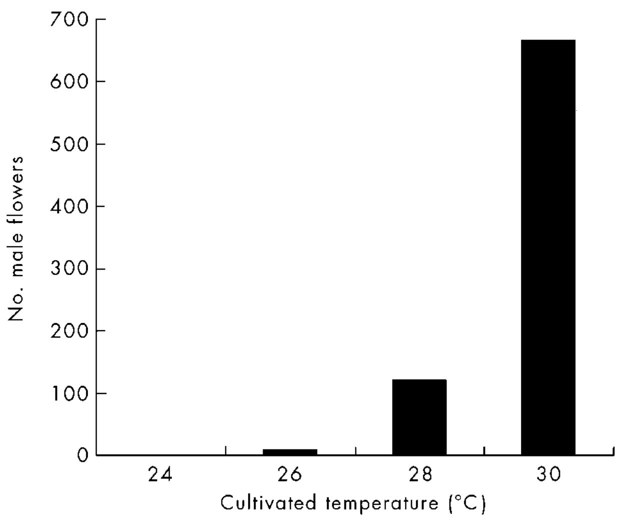

Fig. 3 Average number of male flowers cultivated in incubator of each temperature.

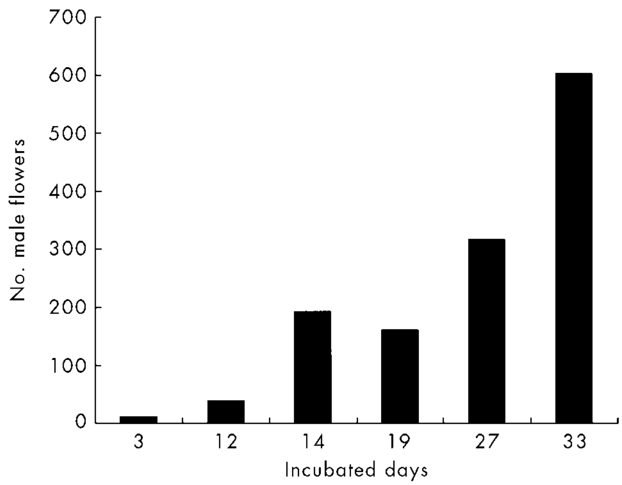

Fig. 4 The effect of duration of male flowers.

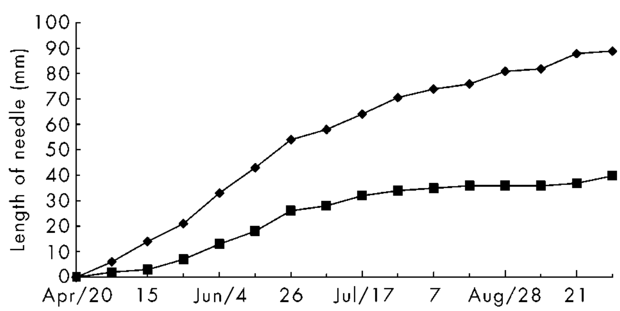

Fig. 5 Difference in elongation of new needle growth between old needles with (■) and without (◆) male flowers in the previous year (i.e. 1989; the measurement year was 1990).

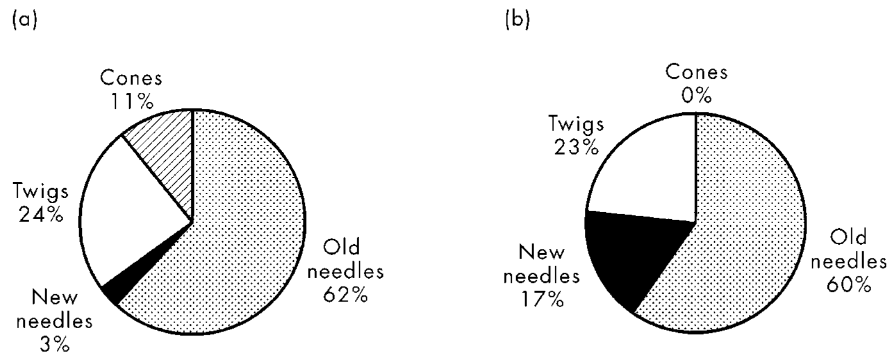

Fig. 6 The percentage of new needles, old needles, cones and twigs in the tree of *C. japonica* with (a) or without (b) male flowers in the previous year (i.e. 1990; measurement year was 1991).

year (Fig. 6). Thus, these ratios showed a significant difference ($P < 0.05$).

Discussion

In *C. japonica* trees, an elongation of new needles begins at the end of April and increases rapidly from May through to July, ceasing by the end of October. The male flower buds are formed in the axil at the apical portion of the new needles.[12] Most male flower buds are formed from late July to late August and grow gradually, maturing by the middle of November. Male flowers bloom with elongation of the floral axis from February to April of the following year.[13,14] After blooming, male flowers drop in June. The female flower buds also form from late July to late August, maturing by the end of October. They bloom from February to April. After pollination, cones gradually grow and mature by the end of October. The cones remain on the trees until the following spring, by which time the seeds have scattered.

In our examination of the differentiation of male flowers, we found that none of the male flowers grew at 24°C incubation. However, under incubating temperatures which ranged from 26 to 30°C, male flowers grew more abundantly as the incubation temperature rose. For the *C. japonica* incubated at 30°C, we found that the longer it was incubated, the more male flowers were produced. The elongation of needles was large in May, June, July and August. However, no male flowers developed until July because the mean temperatures of May (16.7°C) and June (20.5°C) were most likely too low for the differentiation of male flowers. Therefore, male flower differentiation will be accelerated with a larger elongation of needles and the higher temperatures in July.

The correlation coefficients were low between atmospheric pollen counts of *C. japonica* and the mean August temperature ($r = 0.54$; $P > 0.05$), although the mean temperature was higher than that of July. This may be explained by the fact that the elongation of needles in August is less than the elongation of needles in July. Considering temperature conditions for male flower differentiation and elongation of needles, July provides the most suitable conditions for male flower differentiation.[15]

The atmospheric pollen counts of most species were affected by meteorological factors.[16–21] In the case of *C. japonica*, the atmospheric pollen counts had a high positive correlation with the mean temperature in July of the previous year. When the mean July temperatures were above 24.5°C, high atmospheric pollen counts of *C. japonica* were observed in the following year. There is a tendency for high pollen counts of *C. japonica* to appear in two or three year cycles. In 1985, 1988, 1991, 1993 and 1996, when high *C. japonica* pollen counts were recorded, the mean July temperatures of previous years were 25.4°C, 25.4°C, 25.7°C, 24.6°C and 26.9°C, respectively. All of these July temperatures were higher than the average mean July temperature (24.5°C). Several atmospheric pollen counts of *C. japonica* were followed by high atmospheric pollen counts. In 1987, 1989, 1992, 1994 and 1996, when lower atmospheric pollen counts were recorded, mean July temperatures of previous years were 22.2°C, 22.6°C, 24.8°C, 23.2°C and 25.0°C, respectively. This means that July temperatures of previous years, with the exception of those recorded in 1992 and 1996, were lower than the average mean July temperature.

It is thought that cool temperatures in July result in lower atmospheric *C. japonica* pollen counts in the following year. However, in both 1992 and 1996, the previous year's mean July temperatures were 24.8°C and 25.0°C, which were higher than the average mean July temperature. However, atmospheric pollen counts of *C. japonica* were low (Fig. 1). This relationship suggests that factors other than the mean July temperature contribute to male flower production.

Takahashi *et al*. reported similar relationships in the Yamagata prefecture. They estimated the effect of global climate change on Japanese cedar pollen concentration with a formula that used the total pollen counts of previous year as a variable. [22]

The elongation of new needles developed from old needles which produced male flowers was only 44.9% of the elongation of new needles developed from old needles which did not produce any male flowers (4.0 cm/8.9 cm) (Fig. 5).

The increments of new needle weights were significantly reduced to 4.8% (new needle weights (3%)/old needle weights (62%)) of old needles in the trees which produced more male flowers. However, this was 28.6% (new needle weights (17%)/old needle weights (60%)), in the trees that did not produce any male flowers (Fig. 6).

It has been suggested that the productivity of new needles is reduced by the production of male flowers. The reason for this may be that there are other physiological changes occurring in *C. japonica* trees due to the differentiation of male flowers. More particularly, as *C. japonica* trees produce more male flowers and more cones, more of the tree's nutrients are consumed. Therefore, *C. japonica* trees that produce many male flowers are hampered from developing new needles and male flowers in the following year. Consequently, the phenomenon of lower atmospheric pollen counts of *C. japonica* following higher pollen counts, though the mean July temperature remains high, may be explained by changes in the physiological characteristics of these trees.

Yearly atmospheric pollen counts of *C. japonica* show a high correlation with the mean July temperature of the previous year. The yearly mean July temperature varies widely and thus, higher atmospheric pollen counts of *C. japonica* generally appear every two or more years. However, in the year following a rich harvest of male flowers, the number and length of new needles are reduced and male flowers do not develop, despite the mean July temperature being high. We demonstrated that the yearly atmospheric pollen counts of *C. japonica* are affected by physiological changes such as the production of new needles, as well as by the variations in July temperatures of previous years.

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