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Publisher: Taylor & Francis

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Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

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Available online: 05 Nov 2010

To cite this article: Hideaki Taira (2002): Dormancy breaking effects of chilling and altitude on male flowers of *Cryptomeria japonica*, *Grana*, 41:4, 227-231

To link to this article: <http://dx.doi.org/10.1080/001731302321012013>

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Dormancy breaking effects of chilling and altitude on male flowers of *Cryptomeria japonica*

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Taira, H. 2002. Dormancy breaking effects of chilling and altitude on male flowers of *Cryptomeria japonica*. – Grana 41: 227–231. ISSN 0017-3134.

In order to establish precise airborne pollen forecast, these studies were conducted to clarify the dormancy-breaking effects and altitude on male flowers of *Cryptomeria japonica* D. Don. Twigs with male flowers were collected from ten *C. japonica* trees growing on altitudes of 10 m, 200 m, 400 m and 700 m every 10 days between the end of October and early February. Five sets of male flowers from each of these altitudes were prepared for examination. One set was incubated at 10°C and the others were chilled for periods varying from 10 to 40 days at 0°C, and then incubated at 10°C. Chilling was found to release the dormancy of the male flowers, and the effectiveness of the treatment was positively correlated with its duration. Furthermore, the dormancy of the male flowers was also released earlier when the *C. japonica* was growing at high sites, presumably because they were subjected to lower temperatures than those at lower altitudes. Long chilling periods promote the release of dormancy. Knowledge of dormancy better enables the prediction of *C. japonica* pollen dispersal day.

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(Manuscript received 13 August 2001; accepted 13 July 2002)

Pollinosis induced by *Cryptomeria japonica* affects over 10% of the Japanese population and has become a serious health problem in Japan (Inoue et al. 1986). Knowledge of the dormancy and dormancy-breaking characteristics of *C. japonica* flowers is important for predicting when they will begin to disperse pollen and how long pollen dispersal will last. Mizukoshi et al. (1995) reported that symptoms of sufferers who are allergic this pollen improve if they take medicines such as antihistamines before the *C. japonica* pollen dispersal begins. Thus, information on the likely patterns of pollen dispersal is important for prevention and treatment of pollinosis.

Male *C. japonica* flowers grow at the top of the axes of new needles from July through August, and mature by November (Hasizume 1973). Mature male flowers are dormant, but their dormancy is gradually broken by chilling during the autumn and winter (Taira et al. 2000). In an earlier study (Taira et al. 1992) male flowers were found to bloom from the end of February to early March, when the cumulative temperature exceeded threshold levels of 184°C, after which dormancy was completely broken. However, there is the little information about the dormancy-breaking process in male *C. japonica* flowers.

C. japonica is widely distributed on the Japanese archipelago. The northern distribution limit of *C. japonica* is in Aomori prefecture on the main island (40° 42'N), and the southernmost limit of the distribution is Yaku island, of Kagoshima prefecture (30° 15'N). The vertical distribution ranges from an altitude of 0 m in Wakayama prefecture to 2070 m in the Northern Japanese Alps in Toyama prefecture (Taira et al. 1997). Thus, climatic conditions as the range of sites where *C. japonica* grows differ greatly, and would be expected to affect the dormancy breaking process of the

male flowers. Consequently, an examination was conducted to clarify the dormancy breaking effect of chilling and altitude on male flowers.

MATERIAL AND METHODS

The survey was conducted from October 1995 to February 1996 at Toyama prefecture in central Japan (36° 36'N and 137° 40'E; Fig. 1). Sample twigs with male flowers were collected from ten *C. japonica* trees every ten days at altitudes of 10 m, 200 m, 400 m and 700 m. Twigs were cut to a length of 30 cm and placed in a glass tube (2.5 cm in diameter, 12 cm long), which was full of water. The water was changed daily. The twigs from each altitude were divided into five groups. One group was placed in an incubator (LPH-300-RDSMP Incubator, Shimazu Co. Ltd., Tokyo) at 10°C and the others were placed in a refrigerator at 0°C. Every ten days, one group was transferred to an incubator at 10°C, where the light

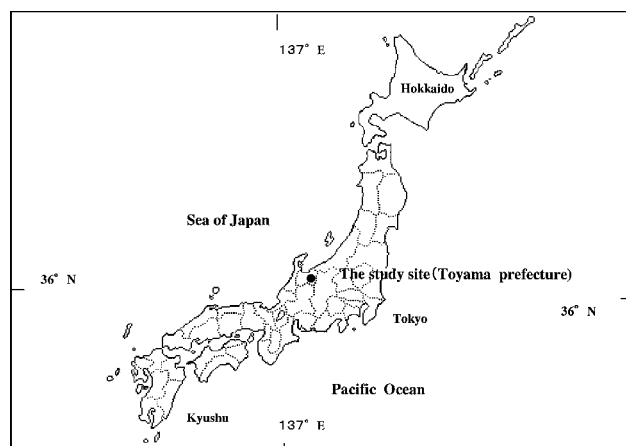


Fig. 1. Location of survey area.

intensity was maintained at 2000 lux for 12 hour/day. Each twig in the incubator was examined daily for pollen emissions until all the male flowers on the twigs had released their pollen.

There are two ways whereby pollen may be released from the male flowers. "Normal" flowering involves elongation of the rachis, which opens the microsporangium, while a second mode involves withering of the male flowers (Taira et al. 2000). The flowers were recorded as blooming on days when all male flowers released pollen. Temperatures at the sites where the plants were growing were estimated from measurements taken at Toyama Meteorological Station, assuming a 0.56°C decrease for each 100 m increase in altitude (Yamagishi et al. 1997).

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

RESULTS

The mean minimum temperature at an altitude of 10 m gradually decreased to under 10°C at the end of October, and then to a low point of less than 0°C at the end of January, although the fall in temperature was not always constant or continuous (Fig. 2). The mean minimum temperature then rose gradually again. At an altitude of 700 m, the mean temperature fell to 10°C about 10 days earlier than at 10 m above sea level. Drops in temperature were consistently earlier as the altitude rose (Fig. 2).

The cumulative temperature needed for the male flowers to bloom at 10 m altitude was 1000°C on 26 October, but this figure gradually decreased to 80°C by 13 February (Fig. 3). On the 26th of October, when the collection of male flowers began, there was little difference in the cumulative temperature needed for flowering at the different altitudes. However, the differences in cumulative temperature needed for blooming at the different altitudes gradually widened, and the maximum margin was recorded on November 26. The difference then gradually decreased again until 13 February, when the cumulative temperature required by the flowers collected from the various altitudes became equal again. There were significant differences ($p > 0.05$) between the cumulative temperatures needed for blooming at altitudes of 700 m, 400 m, and 10 m from 7 December to 15 January. The cumulative temperature needed for male flowers to bloom at an altitude of 200 m tended to be lower than that at 10 m altitude, but there was no significant

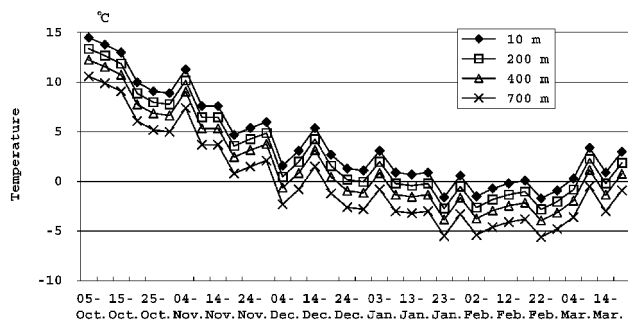


Fig. 2. The mean minimum temperature ($^{\circ}\text{C}$) for every five day period at 10 m, 200 m, 400 m, and 700 m from October 1996 to March 1997.

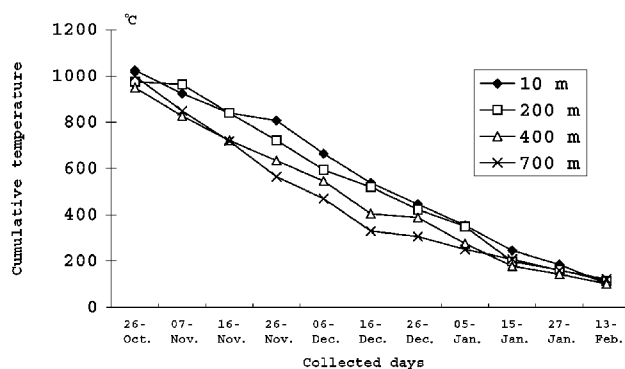


Fig. 3. Cumulative temperature ($^{\circ}\text{C}$) needed for male flowers to bloom at different altitudes.

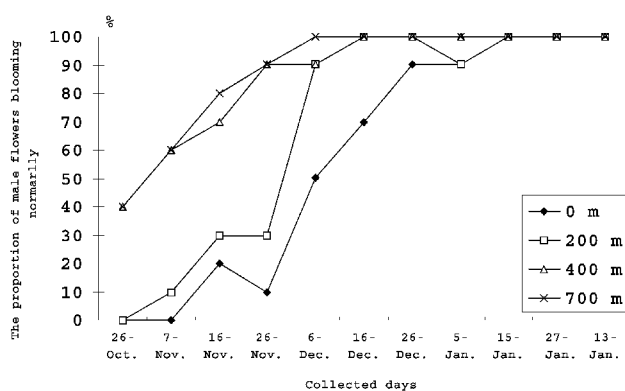


Fig. 4. The proportion (%) of male flowers blooming normally as a function of chilling duration at altitudes of 10 m, 200 m, 400 m and 700 m.

difference ($p < 0.05$) between the altitudes of 10 m and 200 m.

There were no male flowers that bloomed normally with elongation of the axis at 10 m altitude on 26 October and 7 November (Fig. 4). However the proportion of the male flowers that bloomed normally increased gradually after 7 November and all male flowers bloomed normally after January 15. Furthermore, a higher proportion of male flowers growing at high altitudes bloom normally, at early stages, than at lower altitudes. At an altitude of 700 m, 40% of the male flowers bloomed normally on October 26, and all of the male flowers had bloomed by December 6 (Fig. 5).

The cumulative temperature needed for the chilled male flowers to bloom decreased sharply as the duration of the chilling period became longer at an altitude of 10 m from 26th of October onwards. However, the difference in cumulative temperature needed for the male flowers to bloom during the chilling period decreased up to 6 December. Conversely, the cumulative temperature needed for chilled male flowers to bloom increased beyond that required for non-chilled male flowers to bloom as the chilling period lengthened beyond December 6 (Fig. 6). The effectiveness of chilling to reduce the cumulative temperature needed for male flowers to bloom also declined as the

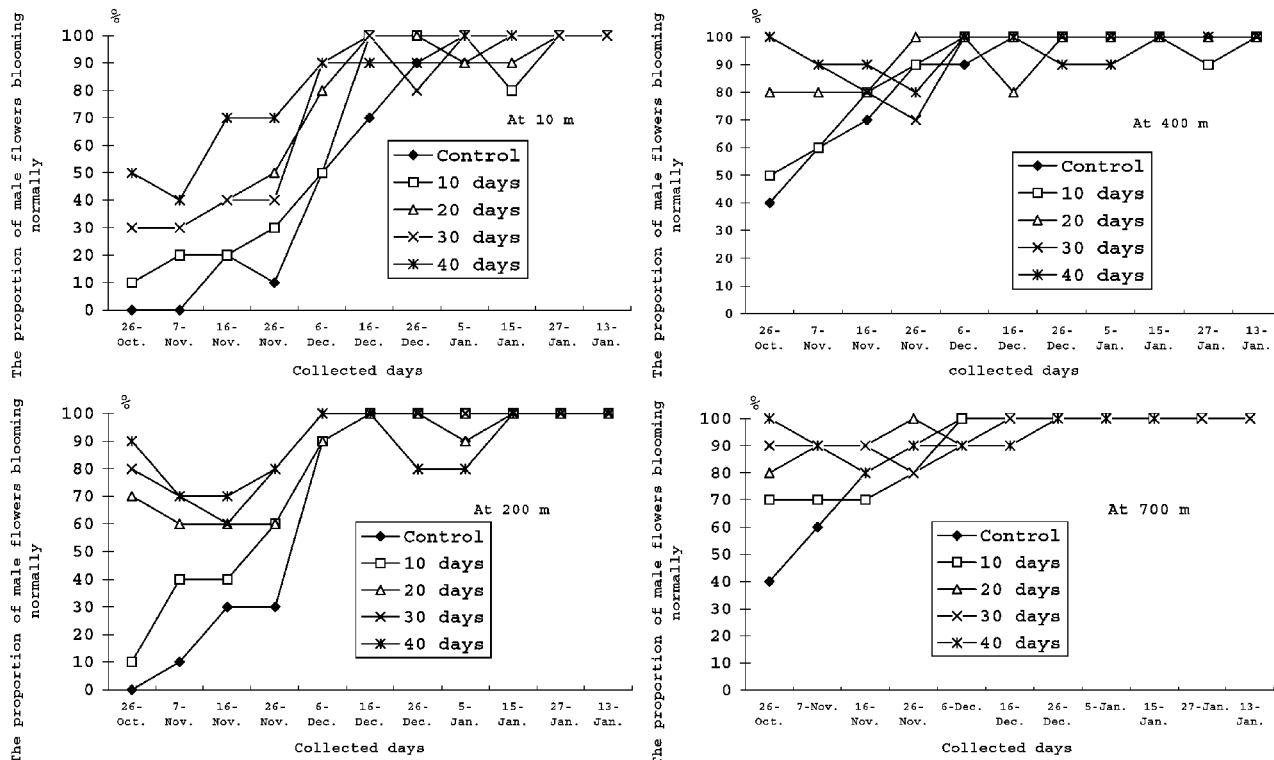


Fig. 5. The proportion (%) of male flowers blooming normally as a function of chilling duration at altitudes of 10 m, 200 m, 400 and 700 m.

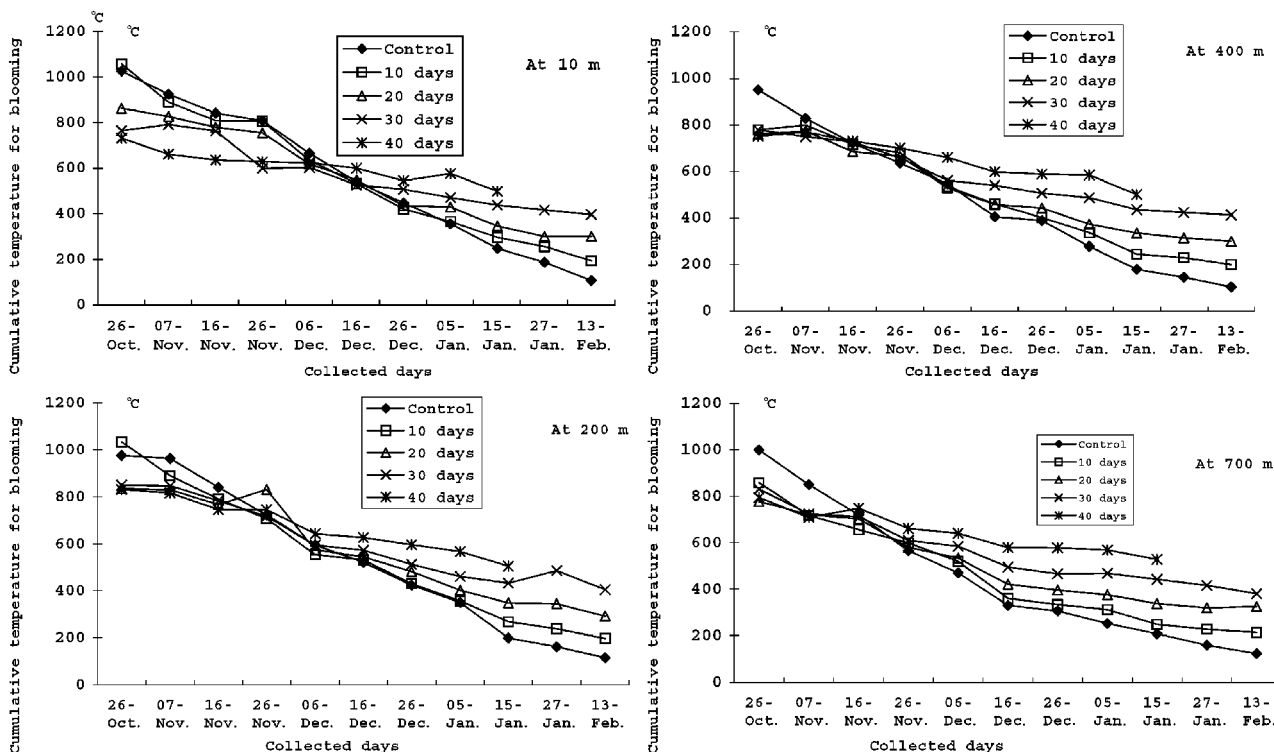


Fig. 6. Cumulative temperature (°C) needed for male flowers to bloom at altitudes of 10 m, 200 m, 400 m and 700 m, as a function of chilling duration.

altitude increased, and there were no differences in the cumulative temperature requirements at 400 m and 700 m altitudes (Fig. 5). The proportion of the male flowers blooming normally increased as the altitude rose, and also as the duration of chilling increased.

DISCUSSION

Most of male flower buds of *C. japonica* are formed from late July to late August, and they continue to develop gradually until the middle of October (Taira et al. 1995). Male *C. japonica* flowers mature between the end of October and November and then remain dormant (Taira et al. 2000). Dormancy serves mainly as a mechanism whereby plants ensure their survival during adverse environmental conditions and synchronizes their growth with the environmental cues, especially when the conditions are favourable (Kobayashi 1987).

The dormancy of the male *C. japonica* flowers is released by chilling during autumn and winter (Taira et al. 2000). The effective temperature range for the releasing dormancy is considered to be 0°C to 10°C, although the most effective temperatures differ according to species (Metzger 1997, Bigras 1997). The mean temperature at 10 m altitude falls to 10°C in early November, and it continues to decrease until the end of January. However, at 700 m altitude, the mean temperature reaches 10°C in mid October; about 10 days earlier than at 10 m. Furthermore, the cumulative temperature needed for the male flowers growing at high altitude to bloom is lower than for those at lower altitudes, and the difference in cumulative temperature requirements between plants growing at 10 m altitude and at higher altitudes was greatest on 28 November. The difference in cumulative temperature requirements between altitudes of 10 m and higher remained large until the end of December. This is because the male flowers growing at higher altitudes are exposed to lower temperatures than those at altitude of 10 m. Therefore, the dormancy of the flowers at 700 m was released earlier than those at 10 m level. The difference in cumulative temperature needed for male flowers to bloom between 700 m and 10 m altitudes gradually narrowed after December, and there was little difference by February 13. This means that the dormancy was released earlier at 700 m than that at 10 m altitude. However, the temperature when dormancy was released at 700 m altitude was lower than that at 10 m. hence, the cumulated temperature needed for male flowers to bloom at 700 m of altitude became the same as for those at 10 m of altitude in February.

The dormancy is released gradually by chilling, and when dormancy is completely over, male flowers bloom usually with elongation of the rachis (Taira et al. 2000). Observations of the male flowers at 10 m altitude showed they had no male flowers that bloomed normally by this mechanism at the end of October or in early November. However, the proportion of male flowers blooming normally increased at the end of November and finally all male flowers bloomed by the end of January. Therefore, the time when the dormancy is completely over appears to be the end of January. These results are consistent with author's previous studies (Taira 2000: unpubl.). On the other hand, at 700 m

above sea level, where the temperature was lower, normal blooming of the male flowers began 20 days earlier than at 10 m altitude and all male flowers bloomed normally by the end of November. This indicates that the dormancy is broken earlier at 700 m altitude than at lower altitudes and, thus, male flowers growing at high altitudes require lower cumulative temperatures for the blooming.

The effects of chilling on breaking dormancy differ according to the length of the chilling period, and they become stronger as the period of chilling becomes longer. A short, 10-day chilling period is ineffective at every altitude. The effects of a long chilling period become more significant at lower altitudes and less significant as the altitude increases. At the altitude of 700 m there was little difference in the effects of the different chilling periods. This is because the dormancy is released earlier at high altitudes than at lower altitudes. At the altitude of 10 m, the cumulative temperature needed for male flowers to bloom decreases as the duration of chilling increases. However, from early December, a long chilling period delays the blooming of the male flowers. This suggests that chilling at 0°C hampers the blooming of male flowers after dormancy is completely released, an effect that appeared earlier as either altitude or the duration of chilling increased.

Crabbe & Barnola (1997) reported that chilling temperatures (0–10°C) have different effects on buds depending on whether they are applied during the phase of increasing growth incapacity (when they increase dormancy) or later (when they reduce it). From observations of male flowers that have bloomed, the dormancy of the male flowers was completely broken by early February, when mean minimum temperatures continued to be around 0°C at 10 m altitude. At 700 m of altitude, all male flowers bloomed normally on December 6 when the mean minimum temperature was around 0°C. Therefore, low temperatures are more effective for breaking the dormancy of *C. japonica* male flowers. However, to confirm which temperature is the most effective, further precise experimentation will be required.

The distribution of *C. japonica* covers a wide range of latitudes, from 40° 42'N to 30° 11'N and the mean annual temperature differs by up to 12°C (Yamagishi 1997). Thus, the time at which dormancy is completely released differs according to the region. Sahashi & Murayama (1993) reported that the pollen front of *C. japonica* differs between observation years. It is because the time of release from dormancy of male flowers change in years and regions. Also, Murayama (1988) and Saito (1985) predicted the day when pollen will be released using a relationship between the mean or maximum temperature in January and the number of days from the 1st of January to the day when pollen was first dispersed. These predictions are prone to considerable error (Taira et al. 1992) because the time that dormancy is released differs among the regions and years. Also, Taira et al. (1994) showed that the cumulative temperature needed for flowering of incubated male flowers after dormancy is completely released, equals the cumulative daily mean temperature from the sample collection day.

Consequently, it is possible to predict the day on which *C. japonica* pollen dispersal will begin by comparing the cumulative incubated temperature for the blooming of male

flowers collected after dormancy is released and the cumulative daily mean temperature from the sample collecting day. However, as the time that dormancy is completely released also differs among years and regions, the optimum day for collecting male flowers precisely when pollen dispersal will begin, should be decided individually for each district.

CONCLUSIONS

In this study, the dormancy of male flowers at lower altitudes was gradually released from early November onwards, and it was completely released by the end of January in Toyama prefecture. The degree of dormancy was affected by the duration of the chilling to which male flowers were exposed. Thus, since male flowers at high altitudes were exposed to chilling earlier, they were released from dormancy earlier than those at lower altitudes. This means that the time of release from dormancy of male flowers differs according to the site at which they grow and climatic conditions prevailing in specific years. Knowledge of dormancy enables us to predict the day on which *C. japonica* pollen dispersal will begin.

ACKNOWLEDGEMENT

The author thanks Mrs. Tomoko Sugimot for her help in laboratory work.

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