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Flower-Bud Formation of Cryptomeria under Controlled Environment*

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Summary In order to elucidate the correlation of temperature and the effect of gibberellin spray on the development of flower-buds in *Cryptomeria japonica* cultivar Kumotooshi, experiments were done in controlled environmental condition during 1970–1971.

The following points were observed:

1. The male flower-buds were formed maximum in the temperature range of 25°C–30°C and their formation decreased with the decrease in temperature. On the other hand, the female flower-buds were formed maximum in the temperature range of 20°C–25°C. At 15°C the both flower-buds were hardly differentiable. At 33°C, they were differentiated, but soon they changed into leaf buds and chlorosis was observed.
2. Male and female flower-buds were formed even at temperatures lower than the optimum ones when sprayed with gibberellin.
3. The effects of the temperature treatment was most predominant at the early term (May 10–Jun. 24) of differentiation in both male and female flower-buds.
4. Under alternating temperature condition the male flower-buds were formed maximum when the mean temperature was around 30°C, and around 20°C, the female flower-buds were formed maximum.
5. Neither male nor female flower-buds were formed at constant temperature treatment (33°C) in the whole period (May–September). However, with a short time treatment at 33°C, the flower-buds were formed in both cases of constant and alternating temperatures.
6. Even at 30°C, the male flower-buds were formed after the samples were treated at the low temperature (chilling) in winter. This result suggests that chilling as a pre-treatment is essential for the flower-bud formation and both adequate temperature and day length conditions can control the flower-bud differentiation period.

Introduction

The flowering habits of the forest trees differ among the tree species and individual trees. In fact, we can find the trees having flowering ages of few years

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and even several decades.

It is well-known that generally even after a tree reaches the flowering age, it has the flowering cycle (fertile or sterile years). These differences are dependent not only upon the genetical implications, but also the effects of different environmental conditions.

Most of the species having flower-bud differentiations are greatly affected by the climatic conditions of the season, especially, the temperature, sunlight and precipitation (Brφndbo 1970, Hasegawa 1943, Ozawa 1962, Sato 1950, Takatoi 1950, Yanagiwara *et al.* 1960).

The following experiments were carried out under the environment-controlled chambers, in order to investigate the effects of temperature conditions upon the flower-bud formation in *Cryptomeria japonica.*

1. The effect of different constant temperatures during the entire period of flower-bud differentiation, from May 10 to September 30.
2. The effect of different constant and alternating temperature treatments during the early, middle and latter terms of the differentiation period.
3. The effect of different constant temperatures after chilling as a pre-treatment.

Materials and Methods

As materials, *Cryptomeria japonica* cultivar Kumotooshi clone was propagated by cuttings in nursery. The experiments were carried out under environment-controlled chamders, Biotron Institute of Kyushu University, during 1970–1971.

Throughout the period of the flower initiation (May 10–Sep. 30), 12 plants per chamber were exposed to different constant temperatures of 15±1°C, 20±1°C, 25±1°C, 30±1°C and 33±1°C. Six plants in all chambers were sprayed with 150 ppm aqueous solution of gibberellin (GA. 3) twice on June 20 and August 3, 1970. 100 ml of 150 ppm aqueous solution of gibberellin were sprayed to each of six plants in all temperature controlled chambers, and control treatment plants were kept outdoors.

The materials were treated at different constant temperatures of 15±1°C, 20±1°C, 25±1°C, 30±1°C and 33±1°C and alternating temperature (the combination between day and night temperature) of 15±1°C/30±1°C, 25±1°C/20±1°C, 30±1°C/25±1°C and 33±1°C/25±1°C in the course of early (May 10–June 24), middle (June 27–August 11) and latter (August 14–September 30) terms. The day temperatures were maintained from 7 a.m. to 7 p.m. and night temperature from 7 p.m. to 7 a.m.. The experimental pots of control treatment and temperature treatments before and after each treatment were kept outdoors.

The chilling pre-treatments were carried out in growth chamber of our laboratory, i. e., under the continuous light with 40W by the plant-lux fluorescent lamp, the materials were constantly treated at 10°C for 3 days from November 10, 1970 at 3 p.m., then at 0°C for 10 days and again at 10°C for 3 days. After these treatments, the materials were immediately placed in phytotron for constant temperature control at 20±1°C, 25±1°C and 30±1°C under natural day length to March 31, 1971. The control treatment pots without chilling were placed in phytotron from November 10, 1970 to March 31, 1971.

Results and Discussions

1. Constant temperature treatment throughout the season of flower-bud differentiation

The whole season of morphological flower-differentiation of *Cryptomeria* is usually from the end of June to the end of September (Hashizume 1968). However, the physiological flower-bud differentiation seems to begin earlier than the morphological differentiation. Therefore, the materials should be exposed to various temperature conditions before the beginning of morphological differentiation. Consequently, the materials were treated at different constant temperature from May 10.

In case of male flowers, the number of clusters, the total number of flower-buds and the number of flower-buds per cluster, and female flowers, only the total number of flower-buds were investigated (Tables 1, 2 and Fig. 1).

(1) The effect of constant temperature treatment

The total number of the male flower-buds seems to have a relationship to the temperature treatment. It decreased as the temperature fell. It decreased in the order of 30°C, 25°C, natural condition, 20°C and 15°C. However, at the temperature of 15°C, the formation of male flower-bud could hardly be recognized. Possibly the differentiation of male flower-buds is repressed at 15°C.

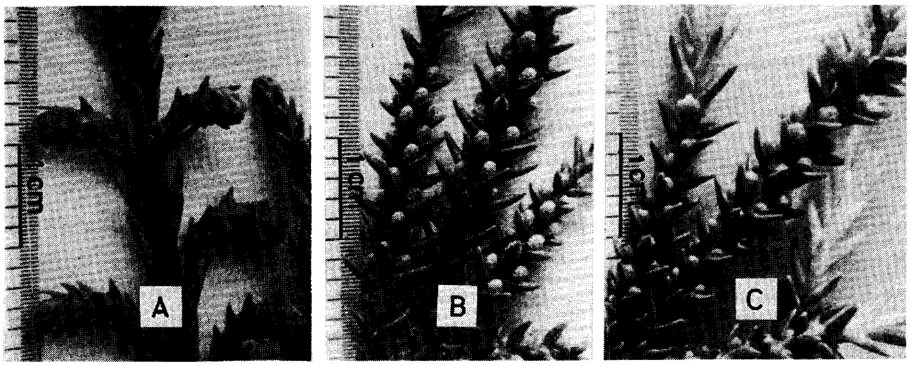

Fig. 1. Effects of the various constant temperature treatments throughout the season of flower-bud differentiation in *Cryptomeria japonica* (May 10 to sep. 30)

A : Female flower buds formed at the constant 20°C

B : Male flower buds formed at the constant 30°C

C : Leaf buds formed at the constant 33°C and chlorosis were observed in new leaves

Table 1. Effect of constant temperature and GA_3 treatments on flower-bud formation in *Cryptomeria japonica*

All plants were placed under natural day length from May 10 to Sep. 30. GA_3 were sprayed twice in 150 ppm. The values are the average of 6 plants.

Gibberellin (GA_3)	Temperature °C	Male flower: No. of buds	Male flower: No. of clusters	Male flower: No. of buds per cluster	Female flower: No. of buds
Treat.	15	115. 3	16. 3	5. 1	25. 2
	20	360. 0	61. 0	7. 2	44. 7
	25	1, 160. 3	66. 0	17. 7	15. 3
	30	2, 877. 5	109. 8	28. 9	4. 7
	33	0. 0	0. 0	0. 0	0. 0
	Natural	2, 958. 7	226. 0	13. 1	31. 0
Untreat.	15	0. 2	0. 2	0. 2	6. 5
	20	24. 5	4. 7	2. 3	21. 7
	25	820. 2	47. 7	20. 0	15. 2
	30	1, 920. 5	64. 2	31. 3	1. 7
	33	0. 0	0. 0	0. 0	0. 0
	Natural	37. 7	5. 5	3. 5	3. 5

Table 2. Analysis of variance of flower buds formed with constant temperature and GA_3 treatments from May 10 to Sep. 30

Source of variation	df	Mean squares: Male flower: a) No. of buds	Male flower: No. of clusters	Male flower: No. of buds per cluster	Female flower: No. of buds
Gibberellin	1	19. 02**	63724. 50**	106. 58 N.S.	2616. 06**
Temperature	5	19. 60**	24663. 46**	1592. 60*	1670. 72**
Gib. ×Temp.	5	3. 37**	19941. 07**	68. 99 N.S.	462. 42**
Error	55	0. 27	545. 07	48. 38	124. 14

a) : calculated by logarithmic transformation of data.
* : significant at 5% level.
** : significant at 1% level.
N.S. : statistically not significant.

At the constant 33°C treatment, chlorosis were observed in new leaves. The leaf-bud development were observed instead of the male flower-buds at the leaf axil.

The number of female flower-buds were maximum at 20°C and decreased in the order of 25°C, 15°C, natural condition and 30°C. However, there were no significant differences among the three temperature treatments of 15°C, natural condition and 30°C. At 33°C constant temperature, no female flower-bud formation was observed.

(2) The effects of gibberellin and constant temperature treatment

After *Cryptomeria* had been treated with gibberellin (GA. 3), the flower-bud differentiation was significantly enhanced in each case.

The total number of male flower-buds were found to be maximum at 30°C and under natural condition; it decreased in the order of 25°C, 20°C and 15°C. In general, at 20°C, 15°C and natural condition, significant differences between GA. 3 treated and untreated materials were observed. At 33° C, normal male flower-buds were not formed and they changed into leaf bud even when sprayed with GA. 3.

The female flower-buds were found to be maximum at 20°C with GA. 3 treatment, and it decreased in the order of natural condition, 15°C, 25°C and 30°C. The promoting effect of GA. 3 on female flower-bud formation was observed at 20°C, 15°C and natural condition. At 33°C, no female flower-bud was formed, even when treated with GA. 3.

In unisexual flowers, both dependence on temperature of flower-bud differentiation and formation is specific to sex. From the experiments with some crops (Itoh 1969, Nitsch *et al.* 1952, Ono 1963), it is reported that, generally, the male flower-bud is inclined to be formed at higher temperature and the female flower-buds at lower temperature.

In the present studies, *Cryptomeria* also revealed same tendency. At the natural day length, the formation of male flower-bud at higher temperature condition tends to be promoted more easily than that of female flower-bud. The male flower-buds were differentiated at the temperature of 30°C and 25°C, but female flower-buds were differentiated at the temperature of 20°C and 25°C. Consequently, the constant temperature treatment of 25°C appears to be the optimum temperature condition of the flower-bud formation in *Cryptomeria.*

The promoting effect of gibberellin on flower-bud differentiation of *Cryptomeria* differs according to variety, tree age, the period of treatment and the times of the treatments. The number of flower-buds in different in these factors (Hashizume 1966, 1968, Shidei *et al.* 1959, 1960).

In this experiment, it was clarified that the effect of gibberellin treatment is dependent upon the temperature. From these results, it can be concluded that differentiation and formation of flower-buds are dependent upon the temperature condition, and when the temperature fell lower than the optimum temperature of each flower-bud formation, gibberellin compensated for the insufficient temperature.

2. Various constant and alternating temperature treatments during different periods

In order to investigate the effects of constant temperature on the flower-bud formation in different period of time, the whole season were divided into 3 periods, called early term (May 10–June 24), middle term (June 27–August 11) and latter term (August 14–September 30) as shown in tables 3, 4, 5 and 6.

(1) The effect of constant temperature

Table 3. Effect of constant temperature treatments during different periods (The values are the average of 6 plants.)

Periods	Temperatures °C	Male flower: No. of buds	Male flower: No. of clusters	Male flower: No. of buds per cluster	No. of female flower-buds
May 10–June 24	15	18.7	4.8	3.1	2.3
	20	387.2	33.0	11.4	12.0
	25	1047.7	67.0	16.1	11.3
	30	1144.7	59.5	18.0	1.5
	33	914.3	60.5	9.4	2.0
	Natural	37.7	5.5	3.5	3.5
June 27–Aug. 11	15	72.7	9.0	4.2	2.6
	20	110.8	13.8	4.0	7.5
	25	257.6	23.0	11.2	2.6
	30	828.8	43.8	21.8	0.2
	33	742.8	38.2	21.1	0.0
	Natural	32.2	3.7	6.6	3.0
Aug. 14–Sep. 30	15	112.7	10.5	7.6	1.9
	20	129.8	12.5	5.7	1.9
	25	294.0	26.0	9.3	5.5
	30	775.3	52.8	15.8	5.2
	33	628.8	53.8	11.8	4.4
	Natural	32.2	3.7	6.6	3.0

Table 4. Analysis of variance of flower-buds formed at constant temperature treatments during different periods

Source of variation	df	Mean squares: Male flower: No. of buds	Male flower: No. of clusters	Male flower: No. of buds per cluster	No. of female flower-buds
Period	2	132220.38*	432.65*	6.20 N.S.	54.89 N.S.
Temperature	5	415962.94**	1339.69**	88.38**	19.88 N.S.
Error	10	30695.32	101.25	15.45	19.92

The male flower-buds were formed maximum at constant temperature treatment of 30°C, and it decreased in order of 33°C, 25°C, 20°C, 15°C and natural condition. The normal male flower-bud formation was not observed when treated throughout the whole season at 33°C. However, with a short constant temperature treatment, i. e., each divided period, normal male flower-buds were formed in all samples. From the view point of treatment periods, except the case of 15°C, all samples reflected the maximum effect of temperature condition in the early term compared with middle and latter terms.

On the development of female flower-buds in early and middle term treatments, Tacle 5. Effect of alternating temperature treatments during different periods

Periods	Temp. day-night °C	Male flower No. of buds	Male flower No. of clusters	Male flower No. of buds per cluster	No. of female flower-buds
May 10- June 24	15—30	785. 7	47. 2	16. 9	14. 7
	20—15	115. 7	12. 0	6. 7	7. 3
	25—20	341. 2	24. 8	10. 1	9. 8
	30—25	1068. 2	50. 2	19. 9	4. 2
	33—25	2346. 8	82. 0	36. 6	2. 8
	Nat.	38. 7	5. 5	3. 5	3. 5
June 27- Aug. 11	15—30	324. 3	20. 3	10. 5	7. 8
	20—15	55. 5	7. 2	6. 2	6. 0
	25—20	158. 8	16. 3	7. 3	8. 0
	30—25	337. 3	27. 5	11. 9	9. 5
	33—25	1144. 0	52. 0	17. 5	0. 2
	Nat.	32. 2	3. 7	6. 6	3. 0
Aug. 14- Sep. 30	15—30	224. 7	18. 5	12. 6	5. 3
	20—15	88. 3	7. 7	5. 8	2. 3
	25—20	259. 8	25. 0	9. 9	8. 0
	30—25	488. 5	38. 0	12. 6	8. 7
	33—25	552. 3	42. 3	12. 8	10. 5
	Nat.	32. 2	3. 7	6. 6	3. 0

Table 6. Analysis of variance of flower-buds formed with alternating temperature treatments during different periods

Source of variation	df	Mean squares: Male flower No. of buds	Mean squares: Male flower No. of clusters	Mean squares: Male flower No. of buds per cluster	Mean squares: No. of female flower-buds
Period	2	457289. 54 N.S.	458. 82*	62. 54 N.S.	2. 56 N.S.
Temperature	5	708454. 53**	1206. 46**	119. 21*	17. 82 N.S.
Error	10	125320. 70	77. 94	26. 50	13. 18

significant differences among various temperature conditions were observed but no significant difference was found in the latter term treatment. In the early term at the temperature of 20°C and 25°C, the middle term at 20°C, and the latter term at 25°C and 33°C, female flower-buds were found. In general, the optimum temperature in the early term (20°C and 25°C) appears to be lower than that of the latter term (30°C and 33°C).

(2) The effect of alternating temperature

The male flower-buds were formed maximum at the day temperature of 33°C and the night temperature of 25°C. The lower the mean temperature was, the fewer the male flower-buds were formed. Remarkable temperature effect was observed in the early term treatment, especially the effect of alternating temperature treatments of 33°C/25°C, and 30°C/25°C was striking.

For the female flower-bud formation, the early term treatment showed the most remarkabe effect at 15°C/30°C, and 25°C/20°C, the middle term treatment at 30°C/25°C. the latter term treatment at 33°C/25°C. To summarize, from the view point of mean temperature, the later the alternating temperature treatment the higher the mean temperature is needed to promote the flower-bud formation, i. e., in the early term; 22.5°C, middle term: 27.5°C, latter term: 29°C.

Generally, the nearer the mean temperature is to each optimum temperature of constant temperature treatments (viz. for male flower-buds 30°C, and for female flower-buds 20°C) the more effective the alternating temperature treatment becomes, otherwise, it is less effective. Furthermore, the effects of the alternating temperature treatment seemed to increase when the night temperature was higher than the day temperature.

It has already been stated that, under the natural condition, the temperature condition of summer time (growing season) is more essential than the other climatic factors on the flower-bud formation of trees. On the study of *Picea abies* (Brøndbo 1970), it was reported that the atmospheric temperature condition from the middle term of June to the early term of July, influenced the flower-bud formation most. In the case of *Cryptomeria*, the temperature treatment in the early term (May 10 to June 24) had maximum influence on the male and female flower-bud formation. Furthermore, it is necessary to examine the combination of temperature in order to clarify the effect of the alternating temperature treatment.

It seems likely from our observation that the flower-bud formation is dependent upon the mean temperature of day and night. The male flower-buds were formed maximum around 30°C of mean temperature, and the female ones around 20°C.

3. The effect of constant temperature treatment after chilling

The samples after chilling started to form the male flower-buds during the end of February at 30°C constant temperature. It was about one and a half months after the new buds had been formed. To examine the conditition of flower-bud formation, at the end of March, all the test pots were taken out from the phytotron.

Table 7 reveals that the male flower-buds were formed only when exposed to 30°C constant temperature after chilling. At the other temperatures, no flower-bud formation was observed. The female flower-bud formation was not observed in any case.

On the basis of these results, for the flower-bud formation of *Cryptomeria*, chilling as a pre-treatment is essential.

From the results mentioned above, it is perceivable that the flower-bud differentiation is not only dependent upon temperature condition but also day length.

Table 7. Effect of constant temperature after chilling on flower-bud formation and length of main shoot

Chilling	Temperature °C	Length of main shoot cm	Male flower: No. of male flower-buds per cluster	Male flower: No. of male flower-buds	No. of female flower-buds
Treated*	20	0. 5	0	0	0
	25	2. 1	0	0	0
	30	3. 2	20. 3	380. 8	0
Untreated (Control)	20	7. 2	0	0	0
	25	13. 0	0	0	0
	30	6. 5	0	0	0

* 10°C / Fluorescent lamp 3 days → 0°C / Fluorescent lamp 10 days → 10°C / Fluorescent lamp 3 days

On the studies of some crops (Itoh 1969, Shisa *et al.* 1966), it was pointed out that day length condition can compensate for the temperature condition, i. e., the higher temperature can compensate for the shorter day length, and the longer day length can compensate for the lower temperature. These observations are consistent with studies on the effect of day length on the flower-bud formation of *Cryptomeria japonica* (Miyajima & Chon unpublished, Migita 1960). It has been known that in summer time (differentiation season) under short day length and at the temperature of 20°C and 25°C, the male flower-buds were hardly formed, and the female flower-buds were formed less than that of natural day length.

These results are suggested that adequate temperature in combination with day length conditions might control the flower-bud differentiation (Fig. 2).

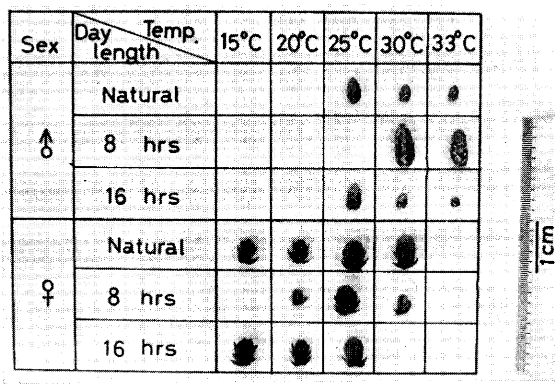

Fig. 2. Forms and Sizes of male & female flower buds formed in various constant temperature treatments under the various day length conditions

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制御環境下におけるスギの花芽形成

宮島　寛・全　尚根

要旨：スギのさし木クローンであるクモトオシの花芽の分化・発達におよぼす温度とジベレリン処理の効果を明らかにするために，1970年5月から71年3月まで環境制御条件下で行なった実験の結果，つぎのことが観察された．

1.　通常の花芽分化期（夏季）において，雄花芽は25°C～30°Cの温度範囲で最も多く形成され，温度の低下につれて減少した．一方，雌花芽は20°C～25°Cの範囲で最も多かった．15°Cでは両花芽とも分化せず，33°Cでは雄花芽は分化したが，間もなく葉芽に転換し，しかも，それはクロロシス現象を起こした．

2.　ジベレリン（GA 3）で処理すると，雌・雄両花芽ともそれぞれの適温より低い温度でも形成された．

3.　温度処理の効果は，雌・雄両花芽ともに分化の初期（5～6月）の処理で最も大きかった．

4.　変温処理の効果は，雄花芽はその平均温度が30°C付近で，雌花芽は20°C付近で最も大きかった．

5.　全分化期間(5～9月）の33°C恒温処理では雌・雄両花芽とも形成されなかったが，短期間の処理では，恒温・変温の両処理ともに効果があった．

6.　冬季の低温（冷温）処理後30°Cの恒温条件下で，3月にはすでに雄花芽の形成をみたが，これらのことから，花芽の形成には前処理として冷温処理が必要であり，しかも適当な温度と日長条件が与えられれば，花芽分化期の調節も可能であるということが示唆された．