

(51) **Effects of chilling treatment on the blooming and the shoot elongation in *Rhododendron indicum* SWEET**

低温処理がサツキツツジの開花とシュート伸長におよぼす影響

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**Abstract** : The seedlings of *Rhododendron indicum* SWEET were chilled at 5°C or outdoor for 40 and 60 days from Dec.4. Then the seedlings with another non chilled seedlings were transferred to two growth chambers which were regulated to 20h day length and kept at 25°C constantly or at 25/18°C alternately (day/night temperature). The other seedlings chilled for 40 days from Nov.27 were grown in two conditions at 15/25°C and 20/25°C (min./max. temperatures). Each area was divided with the curtain; one area was kept on 16h day length (LD) and the other on natural day length (ND). As a result, in non-chilling treatments, about 20% of the flower buds were gradually induced to expand. This means that the flower buds were still in deep dormancy at early winter. They were more induced to open by chilling treatments than non-chilling treatments. So dormancy of flower buds were released by chilled treatments. Blooming under LD was not earlier than ND. The higher the temperature, the earlier the blooming. It is considered that blooming after dormancy released is independent of the photoperiod. There were three regrowing patterns of the terminal flower bud and all of the lateral shoots from the same shoot; all of the lateral shoots started to regrow after the flower bud expansion (Terminal-type), the flower bud started to regrow after all of the lateral shoots finished to elongate (Lateral-type), and both the flower bud and the lateral shoots regrew almost at the same time (Synchronous-type). The Synchronous type is an intermediate pattern between the Terminal and the Lateral types. So the regrowing of them inhibited each other. It is an example of the correlative inhibition phenomenon.

**Key Word** : *Rhododendron indicum* SWEET, Dormancy, Chilling, Blooming, Correlative inhibition

### 1. Introduction

The life cycle of the trees which are growing in forests, parks and streets have influences on growth patterns of individual plants, composition of forests or distribution of the trees<sup>13)</sup>. As the alternating cycles repeat that of nourishment, reproduction and germination, the tree shows different phenologies every year by synchronizing with annual environmental changes; temperature, day length and many external factors.

There are many phenologies to influence the tree distributions, so the life cycles of the trees may be changed by environmental deteriorations such as the earth getting warmer, acid rain and so on<sup>2,3)</sup>. The monitoring of many phenologies in various ways on many tree species in every land, is an important task that will help us to estimate the future forest states and environmental deteriorations<sup>2,3,20)</sup>. In the monitoring of tree phenologies, it is desirable to observe trees with nearly the same heritability. It is important to get enough monitoring data for estimating these effects, so we must perceive many phenologies in large regions and at many sites; schools, governments and so on. Therefore, the plant used for monitoring must be a familiar species with a large sample population.

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It is better that these tree species have hereditary unity, because they will tend to react similarly under the same set of environmental conditions. Although the tree species are not from native seeds, *Prunus yedoensis* has been continued to be observed for the blooming dates in every year since 1953 by the Japan meteorology stations<sup>15)</sup>. *Rhododendron indicum* SWEET cv. *Ohsakazuki* which has been planted at every Japanese regional parks, streets and gardens is proper as a species for observation, too. Because *Ohsakazuki* is a major tree species among Japanese replanted-tree and many seedlings are grown with cuttings from a similar family<sup>12)</sup>.

However, the research mentioning any phenologies of *R.indicum* and *Ohsakazuki* are regarded as few<sup>10)</sup> in comparison to the research about *Populus euroamericana*, *P.yedoensis*, and another evergreen azalea species<sup>5-8,11)</sup>. Consequently, the fundamental data for estimating how environmental changes will affect the life cycles and distributions of living things in the research of *R.indicum* is in short supply. Especially, we have little information on the dormant states of plants which tell us the effects of the earth getting warmer in the winter season.

Therefore, we started to chill the seedlings of *R.indicum* in their dormant state. Further, we warmed those seedlings after the chilling treatment. Then, we investigated the processes of growth of the flower buds and the shoot elongation after the chilling and heating treatments, and considered each relationship. So it was found some phenologies influenced by the dormancy of the flower and the leaf buds in *R.indicum*.

## 2. Materials and methods

### 2.1. Experiment-1

In Experiment-1, we investigated the effect of the winter low temperature condition on the release of the dormancy of the leaf and flower buds of *R.indicum*.

Three years old cutting seedlings potted into the vinyl pots of *R.indicum* cv. *Ohsakazuki* which were grown under outdoor condition in Suzuka City, Mie Pref., Japan, were tested in Experiment-1 (Table 1). The soil condition in the pots was 60% granite soil with 40% compost. Table 1 shows mean size of the seedlings as Experiment-1 begun.

On December 4th, 1991, the test-seedlings were carried to Mie University which is in Tsu City, in the same prefecture. Then, about half number of the seedlings were transferred into the low temperature chamber kept at 5°C constantly and regulated to 24 hours day length. The rest of the seedlings were left under outdoor condition. The seedlings were grown for 40 and 60 days under 5°C and outdoor conditions (chilling treatments). A light intensity in the chamber was about 500lux at the upper part of the seedlings, and the light source was fluorescent lamps.

After that, the seedlings were transferred to the other two chambers kept at 25°C constantly and at 25/18°C (light/dark) alternately regulated to 20 hours day length by lighting with fluorescent lamps (heating treatments). Daily mean temperature in the chamber kept at 25/18°C alternately was 23.5°C during the heating treatment.

In order to judge the depth of the dormancy of flower and leaf buds at starting time of Experiment-1, the other seedlings were transferred to those two growth chambers on December 4th without being chilled (non-chilling

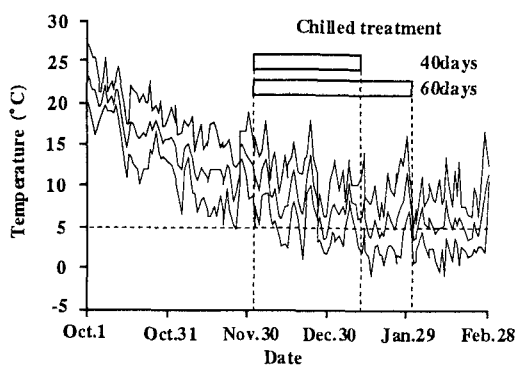


Fig.1 Temperature condition at outdoor during Experiment-1  
Upper line, Max temperature; Middle line, Mean temperature;  
Lower line, Min. temperature

**Table 1** Conditions of each treatment of Experiment-1 (upper) and Experiment-2 (lower) and morphologies of the seedlings<sup>1)</sup>

Chilling treatments <sup>2)</sup>			Heating conditions		Number of flower buds	Seedling height	Seedling spread
Total	( 5℃	Open )	Temperature	Day length			
0day	( 0day	0day )	25℃ <sup>5)</sup>	20h	63.1±17.6	26.7±2.3cm	25.0±3.1cm
0	( 0	0 )	25/18℃ <sup>6)</sup>	20	65.0±13.1	24.6±1.3	21.5±2.2
40	( 40 <sup>3)</sup>	0 )	25℃ <sup>5)</sup>	20	56.8±13.5	26.2±2.1	21.0±2.3
40	( 40 <sup>3)</sup>	0 )	25/18℃ <sup>6)</sup>	20	61.2±11.6	25.7±1.7	21.9±1.6
40	( 0	40 )	25℃ <sup>5)</sup>	20	57.1±15.9	25.2±1.4	20.6±2.7
40	( 0	40 )	25/18℃ <sup>6)</sup>	20	49.3±10.2	28.8±2.4	24.1±1.5
60	( 60 <sup>3)</sup>	0 )	25℃ <sup>5)</sup>	20	54.8±14.5	28.5±2.3	21.2±2.2
60	( 60 <sup>3)</sup>	0 )	25/18℃ <sup>6)</sup>	20	58.1±12.5	25.3±2.1	21.4±1.6
60	( 0	60 )	25℃ <sup>5)</sup>	20	52.6±12.0	26.2±3.1	19.5±2.2
60	( 0	60 )	25/18℃ <sup>6)</sup>	20	65.3±14.4	29.1±4.3	24.7±2.6
40	( 40 <sup>4)</sup>	0 )	Min 20℃ <sup>7)</sup>	16	172.0±14.2	38.7±5.8	46.1±3.8
40	( 40 <sup>4)</sup>	0 )	Min 20℃ <sup>7)</sup>	Natural	193.2±25.7	35.7±1.6	50.2±4.6
40	( 40 <sup>4)</sup>	0 )	Min 15℃ <sup>8)</sup>	16	195.1±20.5	39.8±4.4	52.9±2.3
40	( 40 <sup>4)</sup>	0 )	Min 15℃ <sup>8)</sup>	Natural	173.8±23.0	42.7±2.3	54.9±5.2

1) At the experiments beginning

2) Chilling treatments were begun from Dec. 4 1991 for 40 and 60days in Experiment-1 and from Nov. 27 1991 for 40days in Experiment 2

3) 5℃ and continuous light ( 500lux, top of plants)

4) 5℃ and darkness

5) 25℃ constantly and 20h potoperiod ( 500lux, top of plants at light period)

6) Day and night temperature at 25/18℃ alternately ( 500lux, top of plants at light period)

7) Kept min and max temperature at 20/25℃

8) Kept min and max temperature at 15/25℃

treatments). Thus, the number of treatments of Experiment-1 were as total ten conditions. And we prepared five test seedlings for every treatment (Table 1).

During light period from the sunset to the sunrise, light intensity inside a chamber was over 500lux at the upper part of the seedlings, and the light source was fluorescent lamps. Light periods were from 2 to 8 o'clock and from 16 to 22 o'clock.

Fig.1 shows the temperature conditions at outdoor during an experimental period.

Before the Experiment-1 begun, we optionally selected three flower buds per every seedling. And we put three pieces of the red vinyl tape written to the number-1, 2 and 3 to the shoots having th selected flower bud. We measured lengths of these flower buds and all of the lateral shoots on the same selected shoot by a vernier every 5 days. And we took notes the dates of the phenological changes, such as blooming, sprouting, bud falling off, and so on. The flower bud of *R.indicum* is formed to the top of the shoot<sup>12)</sup>. So the selected flower buds were the terminal buds.

The seedlings were supplied water two times per a day during heating treatments, but were not supplied with fertilizer or agricultural chemicals.

## 2.2. Experiment-2

In Experiment-2, we investigated the effect of the temperature condition after the dormancy released on the regrowth of the leaf and flower buds of *R.indicum*.

Four years old cutting seedlings potted into the vinyl pots of *R.indicum* cv. *Ohsakazuki* which were grown under outdoor condition at Suzuka City, Mie Pref., Japan, were tested in Experiment-2 (Table 1). The soil condition in the pots was peat-moss prepared pH had been 7. Table 1 shows mean size of the seedlings as Experiment-2 begun.

On November 27th, 1991, the test-seedlings were carried to Mie Agriculture Technological Center which is located in a same city. The seedlings were transferred to a low temperature chamber kept at 5℃. And they were grown under the chilled condition for 40 days until January 6th. After that, the seedlings were transferred to two greenhouses kept

**Table 2** Blooming of Experiment-1 (upper) and Experiment-2 (lower)

Chilling treatments <sup>1)</sup>			Heating conditions		Days from the heating treatment beginning			Blooming
Total	5°C	Open	Temperature	Photoperiod	To initiated	To 25% <sup>8)</sup>	To 50% <sup>8)</sup>	Percentage <sup>8)</sup>
0day	( 0day	0day )	25°C <sup>4)</sup>	20h	58.0 <sup>c</sup> ± 5.1	103.0 <sup>ef</sup> ± 20.0		24.0 <sup>c</sup> ± 4.8 <sup>x</sup>
0	( 0	0 )	25/18°C <sup>5)</sup>	20	41.0 <sup>b</sup> ± 4.2	96.0 <sup>ef</sup> ± 15.0		20.8 <sup>c</sup> ± 2.9
40	( 40 <sup>2)</sup>	0 )	25°C <sup>4)</sup>	20	26.8 <sup>a</sup> ± 0.5	33.8 <sup>a</sup> ± 0.9	39.8 <sup>a</sup> ± 0.5	80.0 <sup>a</sup> ± 3.9
40	( 40 <sup>2)</sup>	0 )	25/18°C <sup>5)</sup>	20	26.8 <sup>a</sup> ± 0.5	31.6 <sup>a</sup> ± 1.6	36.4 <sup>a</sup> ± 3.5	59.8 <sup>ab</sup> ± 14.3
40	( 0	40 )	25°C <sup>4)</sup>	20	28.6 <sup>a</sup> ± 2.5	34.4 <sup>a</sup> ± 3.8	43.2 <sup>a</sup> ± 2.5	74.9 <sup>a</sup> ± 5.3
40	( 0	40 )	25/18°C <sup>5)</sup>	20	39.2 <sup>a</sup> ± 1.9	33.2 <sup>a</sup> ± 4.8	42.0 <sup>a</sup> ± 4.0	61.0 <sup>ab</sup> ± 10.5
60	( 60 <sup>2)</sup>	0 )	25°C <sup>4)</sup>	20	28.2 <sup>a</sup> ± 2.8	37.8 <sup>a</sup> ± 3.9	57.5 <sup>b</sup> ± 3.5	63.1 <sup>ab</sup> ± 13.9
60	( 60 <sup>2)</sup>	0 )	25/18°C <sup>5)</sup>	20	31.4 <sup>a</sup> ± 0.9	42.0 <sup>ab</sup> ± 3.8		40.9 <sup>b</sup> ± 13.3
60	( 0	60 )	25°C <sup>4)</sup>	20	32.8 <sup>a</sup> ± 0.9	37.0 <sup>a</sup> ± 3.6	54.3 <sup>ab</sup> ± 6.0	56.3 <sup>ab</sup> ± 16.5
60	( 0	60 )	25/18°C <sup>5)</sup>	20	30.0 <sup>a</sup> ± 1.6	32.0 <sup>a</sup> ± 0.8	41.4 <sup>a</sup> ± 3.7	67.4 <sup>ab</sup> ± 15.9
40	( 40 <sup>3)</sup>	0 )	Min 20°C <sup>6)</sup>	16	49.0 <sup>b</sup> ± 5.7	67.7 <sup>d</sup> ± 2.1	76.2 <sup>c</sup> ± 0.5	82.2 <sup>a</sup> ± 5.4
40	( 40 <sup>3)</sup>	0 )	Min 20°C <sup>6)</sup>	Natural	47.0 <sup>b</sup> ± 1.4	58.0 <sup>c</sup> ± 2.4	78.0 <sup>c</sup> ± 2.4	73.7 <sup>a</sup> ± 3.7
40	( 40 <sup>3)</sup>	0 )	Min 15°C <sup>7)</sup>	16	65.0 <sup>cd</sup> ± 15.1	125.2 <sup>f</sup> ± 5.2	132.4 <sup>d</sup> ± 2.1	74.9 <sup>a</sup> ± 4.1
40	( 40 <sup>3)</sup>	0 )	Min 15°C <sup>7)</sup>	Natural	65.2 <sup>cd</sup> ± 14.1	97.0 <sup>e</sup> ± 4.3	111.6 <sup>d</sup> ± 4.3	75.4 <sup>a</sup> ± 4.5

1) Chilling treatments were begun from Dec 4 1991 for 0, 40 and 60days in Experiment-1 and from Nov 27 1991 for 40 days in Experiment-2

2) 5°C and continuous light (500lux, top of plants)

3) 5°C and darkness

4) 25°C constantly and 20h photoperiod (500lux, top of plants light period)

5) Day and night temperature at 25/18°C alternately (500lux, top of plants at light period)

6) Kept min and max temperature at 20/25°C

7) Kept min and max temperature at 15/25°C

8) Blooming percentages. All of the blooming flower buds ÷ All of the flower buds at the experiments begun × 100

\* a, b, c, d, e, f. Result of *t* test (*p* ≤ 0.05)

min. and max. temperature at 15/25°C or 20/25°C (min./max. temperature). Mean temperature under 15/25°C treatments was 17.8°C and that under 20/25°C treatments was 21.9°C during the heating treatments. Both greenhouses were divided into two sides with the curtain. One sides were regulated long day conditions on 16 hours day length (LD treatments) and the other were not regulated, at all on natural day length (ND treatments) (Table 1). Thus, the number of treatments in Experiment-2 were as total four. And we prepared ten test seedlings for every treatment (Table 1).

During light from the sunset to the sunrise at LD, light intensity inside the greenhouses were over 500lux at the upper part of the seedlings, and the light source was fluorescent lamps. Light periods were from 4 to 8 o'clock and from 16 to 20 o'clock.

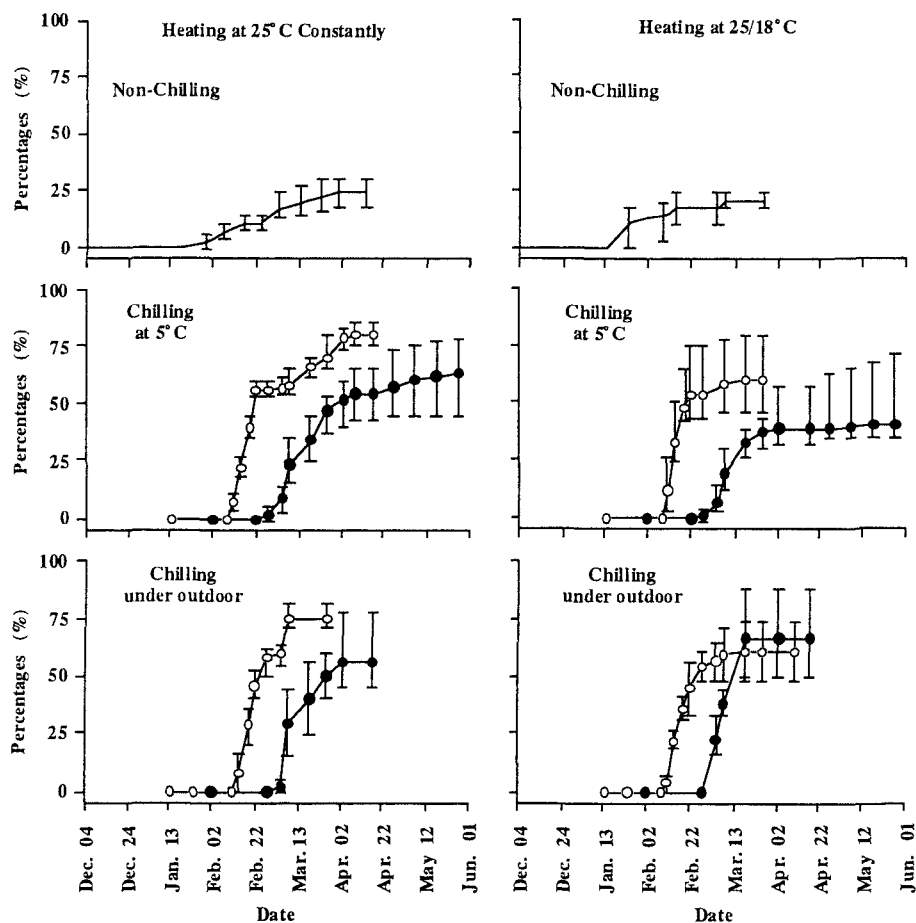
The seedlings were supplied water two times per a day during heating treatments, but were not supplied fertilizer and agricultural chemicals.

## 4. Result and Discussion

### 4.1. Effects of chilling and heating treatments on blooming

In Experiment-1, the flower buds of non-chilled seedlings came into blooming on January 31th that was after 58 days from the beginning of the heating treatment in the chamber kept at 25°C constantly (Table 2). In the other chamber kept at 25/18°C alternately, the flower buds of non-chilled seedlings came into bloom on January 15th that was after 42 days from the beginning of the heating treatments (Table 2). But states of increase of the total blooming flower number under non-chilling treatments were very few, because a lot of flower buds fell off after heated. They were only 20-24% of the end of blooming percentages at all (Table 2).

The flower buds of the seedlings chilled at 5°C for 40 days came into blooming on February 9th that was after 27 days from the heating treatments beginning (Table 2). And states of increase of the total blooming flower number of seedlings chilled at 5°C for 40 days were substantially similar in the both heating chambers (Fig.2). Dates reached to 50% blooming percentages of chilled seedlings at 5°C and outdoor were substantially similar, too (Table 2). Difference of temperature conditions between two chambers did not effect blooming patterns of those seedlings. At all, the total blooming percentages of these seedlings reached to 59.8-80.0% (Table 2). Thus, days from the heating



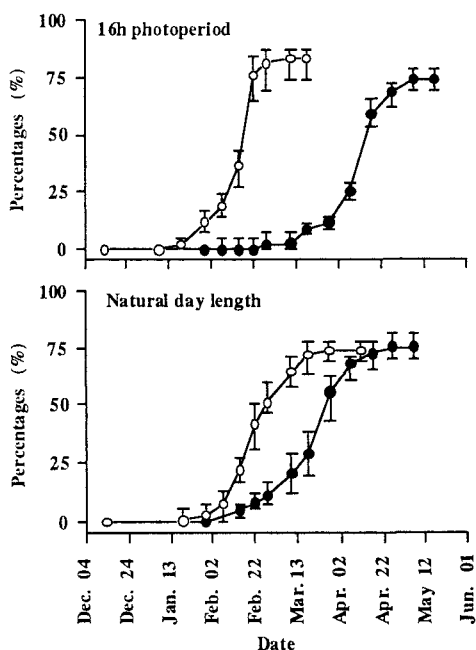
**Fig.2** Effect of the temperature conditions of chilling and heating treatments on blooming of *R.indicum*  
**Chilling treatments**, At 5°C constant of continuous light (500lux, top of the plants) or under outdoor for 40 and 60 days from Dec.4 1991; **Heating treatments**, The plants were grown in the growth chambers adjusted on 20h photoperiod (500lux, top of the plants) and kept at 25°C constantly or day and night temperature at 25/18°C alternately after chilling treatments  
**Open points**, 40 days chilled; **Closed points**, 60 days chilled; **Vertical bars**, Lined from min. to max. percentages

treatments beginning to come into blooming were smaller and blooming percentages were higher in the seedlings chilled for 40 days than those of non-chilled seedlings.

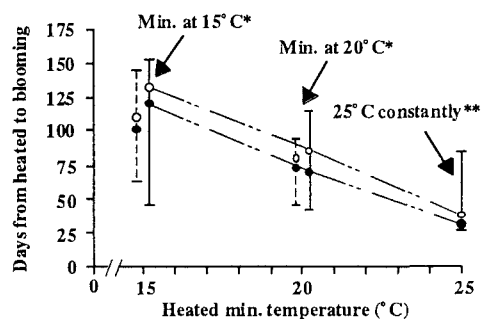
As compared with non-chilled seedlings, blooming patterns of the seedlings chilled for 60 days were conformable at 40 days chilled (Table 2, Fig.2). But it was different from 40 days chilling treatments that the total blooming percentages of three quarters of 60 days chilling treatments were smaller than those of 40 days chilled (Table 2). This reason did not become clear in this paper. Consequently, the flower buds were more induced to expand in chilling treatments than the non-chilling treatments. So dormancy of flower buds were released by chilling treatment for over 40 days from early December. This means that the flower buds were still in deep dormancy at early winter.

#### 4.2. Effects of heating condition on blooming of flower buds

In Experiment-2, the flower buds of 40 days chilled seedlings came into blooming from February 15th to March 10th in the heating chambers. Blooming date under LD conditions was not earlier than that of ND (Fig.3). Thus, the



**Fig.3** Effect of the temperature and photoperiod on blooming of *R.indicum* after the chilling treatment. Chilling conditions, 5°C for 40 days (dark) from Nov 27 1991; Heating conditions, The plants were grown in two greenhouses kept min. and max. temperature at 15°C or 20°C, and max. temperature at 25°C under 16h photoperiod (500lux, top of the plants) or natural day length (500lux, top of the plants). Open points, Min. and max. temperature at 20/25°C; Closed points, Min. and max. temperature at 15/25°C; Vertical bars, Lined from min. to max. percentages



**Fig.4** Effect of the temperature conditions after the winter dormancy released by 40 days chilling at 5°C on the days from beginning of the heating treatments to come into blooming in *R.indicum*

\*, Min. at 15°C or 20°C with Max. at 25°C on 16h photoperiod (500lux, top of the plants) (—) or natural day length (----); \*\*, 25°C constantly on 20h photoperiod (500lux, top of the plants)

Open points, Days reached to 50% of blooming; Closed points, Days reached to 25% of blooming, Vertical bars, blooming periods

LD treatments did not induce to come into blooming and days from the heating treatments beginning to reach 25% and 50% percentages were not different among the same heating conditions (Table 2, Fig.3,4). The LD treatment generally induces to regrow, bloom, sprout and elongate of the shoots in many tree species<sup>13-19</sup>. But the winter buds after dormancy released are not induced to regrow under LD conditions in *Pinus densiflora* and *Populus euroamericana*.

In *R.indicum* it seemed the same reaction as these species. This is considered that the blooming in *R.indicum* are independent of the photoperiod.

In addition to the results of Experiment-1, the days from the heating treatments beginning to come into blooming were the smallest at the treatment of 25°C constantly and 25/18°C alternately, next were at the 20/25°C treatments, and the greatest were at the 15/25°C treatments (Table 2, Fig.4). Thus, the blooming were effected by the temperature conditions after dormancy released. And the higher the temperature, the earlier the blooming.

#### 4.3. Relationships between the regrowth of terminal flower buds and those of lateral buds

In the growth of *R.indicum*, it is said that the blooming period begins from mid. May after finishing of elongation of the "new branches"<sup>12</sup>. The "new branches" are seemed the lateral shoots by the characteristics of morphology of *R.indicum*. It was not clear the relationships between the lateral shoots elongation and blooming of the flower buds from the same shoot elongated in last year.

In Experiment-1, there were three regrowth types of the terminal flower bud and the lateral leaf buds after dormancy released from the same shoot elongated in last year (Fig.5, 6). One type was the "Terminal-type"; the terminal flower bud started to regrow and expanded before the lateral leaf buds started to regrow. Another type was the "Lateral-type"; all of the lateral buds started to regrow, sprouted and elongated before the terminal flower bud started to regrow. The

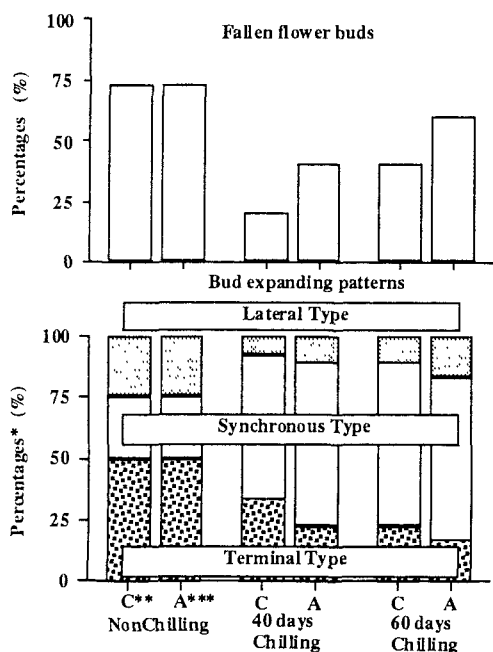


Fig.5 Effect of the days of chilling treatment at 5°C from Dec. 4 on the bud expanding of the terminal flower bud and all of lateral shoots formed at the same shoot of *R.indicum*

\* The percentages of the each patterns among the terminal flower buds which were not abscised; The plants were grown in the growth chambers adjusted on 20h photoperiod (500lux, top of the plants) and kept at 25°C constantly (\*\*) or day and night temperature at 25/18°C alternately (\*\*\*) ; **Terminal type**, All of the lateral shoots started to regrow after the terminal flower bud blooming on the same shoot; **Lateral type**, The terminal flower bud started to regrow after all of lateral shoots finished to elongate on the same shoot; **Synchronous type**, Both of the terminal flower bud and all of lateral shoots on the same shoot started to regrow almost at the same time

other type was the "Synchronous-type"; both the terminal flower bud and the lateral leaf buds started to regrow almost at the same time. Though, it was not clear that the condition of being divided into three regrowth types because there were not enough flower buds for taking statistics some by falling off some flower buds in the experimental periods.

The *Terminal* and the *Lateral* types were considered that the regrowth patterns which starting and regrowing of the terminal or the lateral buds inhibits the other one's regrowth in the same shoot. The *Synchronous* type was intermediate type between them. In general, cause of intermediate phenologies are considered that different phenologies or physiologies inhibit each other<sup>1,15,19-23</sup>. It was an example of the correlative inhibition phenomena. Correlative inhibition are seemed in another plant species, for example, between the growth of the terminal leaf bud and those of the lateral flower buds from the same shoot in *Camellia sasanqua*<sup>20-24</sup>, the elongation and the

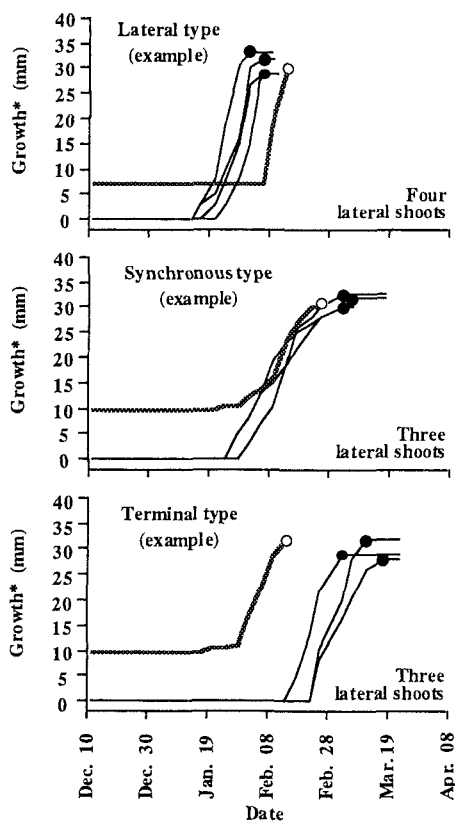


Fig.6 Example of growth of the terminal flower bud and all of lateral shoots formed on the same shoot of *R.indicum*

These plants were grown in the growth chamber kept at 25°C constantly and adjusted on 20h photoperiod after 5°C chilled for 40days from Dec.4 1991

**Open points**, Blooming; **Closed points**, Lateral shoots stopped to elongate; **Wide lines**, Growth of the flower bud length; **Narrow lines**, Growth of lateral shoots

differentiation of the leaf primodias in the same winter bud in *Pinus densiflora*<sup>15,18)</sup>, and the elongation of the plant height and the formation of the aerial tubers in *Begonia evansiana*<sup>1)</sup>.

It was considered that the flower buds come into bloom after finished the shoots elongation in *R.indicum*.. This is Lateral type that is seemed under outdoor condition. In this paper, we seemed the other regrowth patterns in *R.indicum*.

## 5. Conclusions

As a result, it has been clear that effects of chilling treatment on the blooming and the shoot elongation in *R.indicum*.

- 1) Flower bud of *R.indicum* is still in deep dormancy at early winter.
- 2) Dormancy of flower bud is released by the chilling treatment over 40 days.
- 3) The higher the temperature, the earlier the blooming after the dormancy released.
- 4) Blooming after the dormancy released is independent of the photoperiod.
- 5) There is correlative inhibition between the flower bud and the lateral shoot extensions from the same shoot.

Under the condition of the earth getting warmer, the winter chilling for the release of the dormancy of the flower bud in *R.indicum* may be short at the south regions. In this case, the blooming period will be later than now. And it will be changed the life cycles of *R.indicum*, many living things, our sense of seasons and the blooming calender of the park by the chain reaction.

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