Bud burst process and late-frost experiments on *Fagus crenata* and *Quercus mongolica* ssp. *crispula*

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Bud burst timing and frost tolerance were examined in *Fagus crenata* Blume and *Quercus mongolica* Fisscher ex Ledeb ssp. *crispula* (Blume) Menitsky, which are dominant species in the cool-temperate forest zone in Japan. The northern limit of distribution of the former species is within Kuromatsunai, Hokkaido and the latter extends its territory throughout the northeast and interior of Hokkaido. The temperature dependence of the bud burst process was observed in Sugadaira Heights, Nagano Prefecture, for seven years. An experiment on frost effects on leaves for both species was conducted. *Fagus crenata* initiated bud burst earlier than *Q. mongolica* ssp. *crispula* in spring, although a strong thermal dependence of the bud burst process was observed in both species. The first leaf unfolding stage occurred on 12 May for *F. crenata* and on 27 May for *Q. mongolica* ssp. *crispula*. The average thermal times (TTs) for the leaf unfolding stage were 113±11°C day for *F. crenata* and 182±16°C day for *Q. mongolica* ssp. *crispula*. Experiments on freezing tolerance of winter buds and unfolded buds were carried out using a freezer and a natural-radiative-cooling box, which was newly devised for this experiment. The winter buds and buds in swelling stage were tolerant to freezing but buds in the leaf unfolding stage and the new shoot stage were severely damaged and were unable to recover. Shoots of *Q. mongolica* ssp. *crispula* formed lateral buds, which developed leaves if apical buds were damaged. These results suggest that *F. crenata* may not be able to disseminate in regions which may experience frost after the TT of a 113±11°C day has occurred, but *Q. mongolica* ssp. *crispula* can disseminate due to its delayed bud burst date and can regenerate shoots from lateral buds.

**Key words**: bud burst time, *Fagus crenata*, frost experiment, geographical distribution, *Quercus mongolica* ssp. *crispula*

**Introduction**

*Fagus crenata* and *Quercus mongolica* ssp. *crispula* are representative tree species in the cool-temperate forest zone in Japan. The geographical distribution of both species overlaps from the south island (Kyushu island) to the north island (Honshu island). However, the distribution of both species in Hokkaido is different. The northern distribution limit of *F. crenata* lies around the Kuromatsunai Lowland in Oshima Peninsula, the southwestern part of Hokkaido (42°40′N, 140°18′E), while *Quercus mongolica* ssp. *crispula* is distributed almost throughout Hokkaido (Horikawa 1977). The northern limit of distribution in *F. crenata* has been discussed by a lot of researches (Honda 1922; Kira 1947; Tsukada, 1982; Watanabe 1994 and so on).

Kira (1947) reported that the warmth index (WI) and coldness index (CI) which are cumulative temperature indices can explain vertical and horizontal distributions of woody plants in Japan. *F. crenata* and *Q. mongolica* ssp. *crispula* are distributed in regions within the WI between 45 and 85°C month. This implies that both species have similar temperature requirement to grow and reproduce. However, it seems that the WI cannot completely explain the distribution range of natural populations of *F. crenata* (Kira et al. 1976), because *F. crenata* is not distributed...
in the northeastern part of Hokkaido, the WI of which should enable *F. crenata* populations to disseminate (Horikawa 1977; Watanabe 1994). Wada (1983) mentioned the distribution of *F. crenata* population to the lowland and montane regions in Nagano prefecture, Honshu Japan in relation to Kira’s warmth index and snow.

In general, the northern distribution limit of plants depends on their ability to adapt eco-physiologically to low temperatures (Woodward 1986; Mariko et al. 1993). Sakai (1975) determined the freezing resistance of trees growing under different cold climates. The freezing resistance of *F. crenata* and *Q. mongolica* ssp. *crispula* buds was similar because the threshold temperature were −27 °C for *F. crenata* and −30°C for *Q. mongolica* ssp. *crispula* (Sakai 1982).

The mean minimum air temperature in winter does not reach not below −30°C even in the northeastern part of Hokkaido (Kira et al. 1976). Therefore, the WI and freezing resistance of winter buds cannot explain the absence of *F. crenata* populations in northeastern parts of Hokkaido.

Elsewhere late frost is one of the thermal–meteorological factors and has an instantaneous impact not only on leaf survival but also on plant survival (Sakai 1982). According to a long-term observation in Sugadaira Heights, Nagano Prefecture, *F. crenata* starts leaf unfolding of winter buds earlier than *Q. mongolica* ssp. *crispula* (Hayashi, unpublished). This observation suggests that *F. crenata* may suffer more frequent and serious damage due to exposure to late frost than *Q. mongolica* ssp. *crispula* (Kaji & Takahashi 1999; Kuroda et al. 2001). Hayashi (1988) also pointed out that the northern limit of *F. crenata* passes includes regions where the latest frost occurred between 10 and 20 May. Kashimura (1978) observed that *Q. mongolica* ssp. *crispula* has a stronger ability to recover from freezing damage to shoots than *F. crenata*, and insisted that this explains the difference in local distribution pattern of both species in snowy mountains. These findings imply that the latitudinal distribution of *F. crenata* populations may depend on the timing of bud burst and the latest frost. Thus, the relationship of the timing of bud burst and late frost (the bud burst–frost hypothesis) is a reasonable explanation for the geographical distribution as compared with the cumulative temperature. However, the bud burst–frost hypothesis for the geographical distribution of *F. crenata* and *Q. mongolica* ssp. *crispula* has not been tested with experiments.

The present study aims to test this hypothesis by obtaining related data. The bud burst date of *F. crenata* and *Q. mongolica* ssp. *crispula* was observed for seven years in the field and the freezing tolerance of buds in bud burst process was experimentally examined.

Materials and Methods

Definition of bud burst process

The bud burst process is divided into six stages from the winter bud stage (L0) to the full expanding stage (L5) based on the modified Watanabe’s classification (Watanabe 1987). Each stage is described as follows (Fig.1):

- **L0**: winter buds
- **L1**: a bud swells and its end splits so that green tissues are exposed
- **L2**: distal end of leaf opens and leaf unfolding begins
- **L3**: first leaves opens and a clear shape can be identified
- **L4**: second leaves begin unfolding
- **L5**: all leaves completely unfolded and maximum leaf area attained.

The term “bud stage” indicates L0–2 and “new shoot stage”, L3–5, which was judged based on the above criteria. The definition of the bud burst process is applied to an entire tree in field observations and a given bud or new shoot in frost experiments.

Field observation on bud burst process and thermal environment in Sugadaira Heights

Field observations of the bud burst process in adult trees of *Fagus crenata* and *Quercus mongolica* ssp. *crispula* were carried out in the arboretum of the Sugadaira Montane Research Center (SMRC, 1320 m a.s.l., 36°31′N, 138°21′E), University of Tsukuba, in April, May and June for seven years (1988–1994). We record the bud burst time of a marked tree for each
The stages in leaf unfolding process in the case of *Fagus crenata*.

**L1**: a bud swells and its end splits so that green tissues are exposed

**L2**: distal end of leaf opens and leaf unfolding begins

**L3**: folded of first leaves opens and a clear shape can be identified

**L4**: second leaves begin unfolding

**L5**: all leaves completely unfolded and maximum leaf area attained.

**Fig. 1.** The stages in leaf unfolding process in the case of *Fagus crenata*.

species every day during the observation period in order to estimate the thermal time. Thermal time (TT, °C day) is the sum of daily mean air temperature above 5°C until the bud burst process of both species reached each stage of L1 to L5.

\[
TT = \sum (tm - 5)
\]

where \( n \) is days with daily mean air temperature higher than 5°C and \( tm \) is the daily mean air temperature (°C) on those days. The thermal time was calculated from the data of air temperature (120 cm above the ground level) recorded at the meteorological stations in the SMRC between 1988 and 1994.

**Freezing experiments**

In order to examine the frost tolerance of buds and new shoots, experiments on freezing damage were
conducted using a freezer (indoor experiment) and a natural–radiative-cooling box developed by Hanyu et al. (1979) (outdoor experiment). It is expected that the degree of damage from frost depends on the stage of the bud burst process. Seedlings planted in pots were used for freezing experiments. *F. crenata* seedlings were collected from a beech forest in Kayanodaira, Nagano Prefecture on 28 October 1993, and *Q. mongolica* ssp. *crisputa* seedlings were collected from the arboretum of SMRC, University of Tsukuba, on 29 October 1993. Three-to-five-year-old seedlings were planted in pots (18 cm in diameter). Five replications were made for the experiment. The number of seedlings per pot was one or two for each species. The seedlings used for freezing experiments had shoots at stages L1, L2 and L3. The freezing-treated shoots were visually judged to be dead or alive two months after freezing: "Dead," all leaves were black and wilted; "Alive," leaves were partially or entirely of normal color and not wilted.

In the indoor frost experiment using the freezer, the air temperature was controlled between a room temperature and −7°C. The air temperature in the freezer was gradually decreased from room temperature to −7°C at a rate of 5°C per 20 minutes shown in Fig. 2. It took 100 ± 10 minutes to decrease to low temperatures below −5°C. After freezing for about 200 minutes, the temperature was gradually increased to a room temperature at a rate of 5°C per 20 minutes. The seedlings were kept in the freezer for 400 minutes. The seedlings were previously moistened with a water sprayer in order to maintain a frosty condition, avoiding super cooling (Kitaura 1967; Hanyu et al. 1986). The seedling pots placed in the freezer were covered with 5-cm-thick Styrofoam boards to avoid soil freezing. During the experimental period, the time courses of air, bud and leaf temperatures were recorded every 30 seconds using thermocouple thermometers and a data logger (EKO Instruments Trading, SOLAC III). The changes of leaf temperature of both *F. crenata* and *Q. mongolica* ssp. *crisputa* pursued almost the same time course.

The seedlings of *F. crenata* and *Q. mongolica* ssp. *crisputa* were placed in the natural–radioactive-cooling box (90 cm in width, 180 cm in long, 45 cm in depth) in which the air temperature can be decreased.

**Fig. 2.** Changes in air temperature (○), swelling bud L1 (×), leaf temperature in L2 (●) and L3 (▼) stages of *Qurecus mongolica* ssp. *crisputa*. The arrows show the time when temperature in the freezer were operated (decreased or increased). The leaf temperatures of L1 and L3 stages were not distinguished because these pursued almost the same course.
to a temperature lower than that of ambient air by natural radioactive cooling (Hanyu et al. 1979). The top of the box was covered with two sheets of polyethylene film, and the four sides and the bottom of the box were insulated with 10-cm-thick Styrofoam boards. The inside Styrofoam board surfaces of four sides were covered with 15-μm-thick aluminum foil and the inside surface of the bottom was painted black in order to reduce long-wave radiation emitted from the aluminum foil surfaces (Fig. 3). The seedlings were sufficiently moistened with the water sprayer. The seedling pots were surrounded with 5-cm-thick Styrofoam boards. The box was placed on the asphalt ground from sunset until sunrise. During the experimental period, the time courses of air temperatures inside and outside of the box, and the bud and leaf temperatures were recorded every 30 seconds using the thermocouple thermometers and the data logger (Fig. 4).

The significant difference in freezing damage between the stages of the bud burst process was tested by Fisher's exact probability test at $P=0.05$.

**Fig. 3.** Natural-radiative-cooling box in the outdoor frost experiment. A: parts and structure of the box, B: cross section of the box.

**Fig. 4a.** Comparison of air temperature in (Tb) and out of (To) the natural-radiative cooling box on clear and windless days. (a) 18-19 Feb. 1994, (b) 21-22 April 1994, (c) 19-20 May 1994

**The warmth index and late frost in southern Hokkaido**

Air temperatures and late frosts in Iwamizawa, Sapporo, Kutchan, Sittu, Tomakomai, Muroran, Esashi and Kuromatunai were examined with the Table of Japan Meteorological Agency (1991).
Fig. 4b. Changes in air temperature and temperature of swelling bud (L1), unfolding bud (L2) and unrolled leaf (L3) in *F. crenata* seedlings in a natural-radiative-cooling box on a clear and windless day (19-20 May 1994).

### Table 1.
The thermal time (TT) with standard deviation in parentheses and the date at each bud burst stage of *Fagus crenata* and *Quercus mongolica* ssp. *crispa*

<table>
<thead>
<tr>
<th>Bud burst stages</th>
<th><em>F. crenata</em></th>
<th><em>Q. mongolica</em> ssp. <em>crispa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.T.</td>
<td>Date</td>
</tr>
<tr>
<td>L1</td>
<td>66.2 (18.4)</td>
<td>2-6 May</td>
</tr>
<tr>
<td>L2</td>
<td>99.2 (16)</td>
<td>9-14 May</td>
</tr>
<tr>
<td>L3</td>
<td>113.2 (11.4)</td>
<td>11-20 May</td>
</tr>
<tr>
<td>L4</td>
<td>131 (17.2)</td>
<td>15-21 May</td>
</tr>
<tr>
<td>L5</td>
<td>175.2 (16)</td>
<td>24-29 May</td>
</tr>
</tbody>
</table>

### Table 2.
Frost damage to different bud burst stages in *Fagus crenata* and *Quercus mongolica* ssp. *crispa* in the freezer and natural radiative box.
L1, L2 and L3 are buds in swelling stage and new shoot stage.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Live(%)</th>
<th>Dead(%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. crenata</em></td>
<td>L1&amp; L2</td>
<td>88</td>
<td>12</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>11</td>
<td>89</td>
<td>**</td>
</tr>
<tr>
<td><em>Q. mongolica</em> ssp. <em>crispa</em></td>
<td>L1&amp; L2</td>
<td>45</td>
<td>55</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>9</td>
<td>91</td>
<td>**</td>
</tr>
</tbody>
</table>

**Buds in Freezer**

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Live(%)</th>
<th>Dead(%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. crenata</em></td>
<td>L1&amp; L2</td>
<td>100</td>
<td>0</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>38</td>
<td>62</td>
<td>ns</td>
</tr>
<tr>
<td><em>Q. mongolica</em> ssp. <em>crispa</em></td>
<td>L1&amp; L2</td>
<td>100</td>
<td>0</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>80</td>
<td>20</td>
<td>**</td>
</tr>
</tbody>
</table>

**: significant at <0.05, ns: not significant
RESULTS

Thermal effects on bud burst process in Sugadaira Heights

A daily mean air temperature above 5°C was recorded after 24 March on the average between 1988 and 1994. Buds in the L1 stage were observed on 2–6 May (TT, 66.2°C day) for Fagus crenata and on 4–8 May (TT, 54.1°C day) for Quercus mongolica ssp. crispula (Table 1). The stage of first leaf unfolding (L2) occurred on 9–12 May (TT, 99.2°C day) for F. crenata and on 12–28 May (TT, 156°C day) for Q. mongolica ssp. crispula. F. crenata and Q. mongolica ssp. crispula unfolded leaves (L3) on 11–20 May (TT, 113°C day) and on 22 May–1 June (TT, 182°C day), respectively. F. crenata unfolded the leaves earlier than Q. mongolica ssp. crispula. The leaf unfolding stage of L5 was attained on 24–29 May in F. crenata and on 9–14 June in Q. mongolica ssp. crispula.

Freezing experiments

The bud and leaf temperatures in the freezer were higher than the surrounding air temperature throughout the experimental period, during which the air temperature was decreased from room temperature to −7°C and then increased back to room temperature (Figs. 2 and 4). The new leaves (L3 stage) were exposed to temperatures lower than the ambient temperature but the temperature of buds at L1 and L2 stages was always higher than the air temperature. The difference in air temperature was larger in new leaves than in winter buds and swelling buds. Eighty-eight percent of buds in the L1 and L2 stages of Fagus crenata survived, while eighty-nine percent of leaves in the L3 stage were injured and died (Table 2). The difference in freezing injury rate between the buds and new shoots was significant (P <0.05). In the case of Quercus mongolica ssp. crispula, there was no significant difference in freezing tolerance between the buds and new shoots.

The temperature in the natural–radiative–cooling box was 7–10°C lower than the outside air temperature overnight on clear and windless days. Fig. 4a shows the typical time courses of air temperature inside and outside the natural–radiative–cooling box on clear days, 19–20 May 1994. The natural–radiative–cooling box had the lowest air temperature of approximately −8°C when the outside air temperature was about 0°C. This difference remained the same between 2:00 and 4:00. There was a trend that the unfolded leaf temperature of F. crenata seedlings was lower than the air temperature in the box by 1–1.5°C (Fig. 4b). The temperatures of swelling buds (L1) and unfolding leaves (L2) were higher than those of surrounding air and new leaves by 1–2°C. Natural–radiative cooling caused no damage to the L1 and L2 buds of F. crenata and Q. mongolica ssp. crispula seedlings (Table 2). The new shoots in the L3 stage were injured; 62% for F. crenata and 20% for Q. mongolica ssp. crispula (P <0.01), respectively.

Discussion

Thermal effects on bud burst process

From the viewpoint of matter production by photosynthesis, earlier occurrence of bud burst is advantageous for temperate trees, because they can initiate earlier matter production in developing and developed leaves. At the same time, however, the risk of exposure to late frost for the developed leaves exists at the beginning of the growing season. The timing of bud burst in temperate trees is decided taking into consideration the risk and benefit. On the other hand, the bud burst process is greatly dependent on temperature (Cannell & Smith 1983). In most temperate-zone trees, bud burst timing has been related to the previously accumulated amount of heat above a threshold temperature (Wang 1960). Heat units such as thermal time have been variously modified by winter chilling, photoperiod, soil temperature, and various plant characteristics (Campbell & Sugano 1975, 1979; Campbell 1978; Lavender 1980; Thomson & Moncrieff 1982).

The present study demonstrated that the winter buds of Fagus crenata and Quercus mongolica ssp. crispula trees developed with the increase in the thermal time in spring (Table 1), indicating the strong thermal dependence of the bud burst process in both species. The development of buds and new shoots of F. crenata trees was more rapid than that of Q. mongolica ssp. crispula trees which grew in the same place, although
their canopies receive the same amount of thermal time in Sugadaira Heights. Maruyama (1979) reported that the bud burst of F. crenata preceded that of Q. mongolica ssp. crispula in the tallest tree layer in the summer-green forest. These results reveal that F. crenata trees are more sensitive to temperature than Q. mongolica ssp. crispula ones.

Freezing injury

The experiment demonstrated that buds in the L1 and L2 stages were tolerant to frost but leaves in the L3 stage were severely damaged (Table 2). The new shoots after the L3 stage in both Fagus crenata and Quercus mongolica ssp. crispula must be severely damaged when they were exposed to late frost in their habitats (Kashimura 1978). The freezing tolerance of swelling buds and unfolding leaves may be attributable in part to an ability to maintain their temperatures higher than the air temperature, which was supported by experiments using the freezer and the natural-radiative-cooling box (Figs. 2 and 4). The temperature of new leaves was higher in the freezer and lower in the natural-radiative-cooling box than the surrounding air temperature. The freezing intolerance of the new leaves is due to the decline in physiological abilities to tolerate freezing (Sakai 1982). The decrease in leaf temperature in the natural-radiative-cooling box is due to radiative heat loss and is in accordance with that outside the box; but the mechanism of decreasing temperature is different from that in the freezer, in which the leaf is directly cooled by cold air (Hanyu et al. 1978). Therefore, the natural-radiative-cooling box provides more useful results of freezing experiments than the freezer. The outdoor freezing experiment demonstrated that the new leaf temperature must decrease lower than the outdoor air temperature (Fig. 4b).

Q. mongolica ssp. crispula has terminal and lateral buds on the top of a shoot as a shoot-forming system. Kashimura (1978) reported that the lateral buds of Q. mongolica ssp. crispula fell without leaf formation when the terminal buds normally unfolded, but, when terminal buds were damaged, the lateral buds grew into new leaves. The same result was observed in both the freezer and natural-radiative-cooling box experiments (Kojima 1994). Such a bud burst strategy of Q. mongolica ssp. crispula seems to be an avoidance strategy for cold stress (Fitter & Hay 1981; Kashimura 1978). However, F. crenata has not acquired the same strategy for avoidance because of the absence of lateral buds. Therefore, F. crenata is unable to recover via the lateral buds when terminal buds were injured by frost. In this context, it is expected that F. crenata suffers more seriously from exposure to late frost than Q. mongolica ssp. crispula (Kashimura 1978).

The winter buds of F. crenata required the thermal time of a 113°C day to reach the L3 stage in Sugadaira Heights (Table 1). Therefore, frost occurring after a 113°C day will severely damage F. crenata. In other words, F. crenata is unable to survive in an area where late frost occurs after the 113°C day has occurred, because their leaves are injured by late frost without being able to recover via alternative buds.

Bud burst time late frost hypothesis

The areas in southern Hokkaido where the late frost occurs after thermal time of a 113°C day are shown in

Fig. 5. Map showing the locations which thermal days and late frost time were examined in southern Hokkaido. Open circles are the sites which have late frost before the thermal days of 113°C day and solid circles are the sites the late frost occurs after thermal day of 113°C day.
Fig. 5. The cities of Kuromatsunai, Suttsu, Muroran, Esashi, Tomakomai and Sapporo are safe sites for *F. crenata* to survive but Kutchian and Iwamizawaz are impossible to survive because the late frost occurs after the thermal day of a 113°C day.

Between Kuromatsunai and Kutchian, there are mountains such as Mt. Raidenyama, Mt. Youteizan, Mt. Nisekoanupuri, Mt. Konbutake, Mt. Usuzan and Mt. Tarumaeyama in which are supposed to have the late frost after a 113°C thermal day. Therefore, *Fagus crenata* hardly disseminates to the mountain areas. The geographical line along which frost occurred after the days amounted to 113°C day, passes through areas at near the Kuromatsunai region, Southern Hokkaido. On the other hand, *Quercus mongolica* ssp. *crisipula* avoids the risk of frost through delayed leaf unfolding and lateral buds, which form new leaves when terminal buds were injured. Thus, the northern limit of distribution of *F. crenata* can be indeed explained by the bud burst time–frost hypothesis (BB–LF hypothesis). According to the BB–LF hypothesis, it is expected that *F. crenata* is able to grow even in the northeastern Hokkaido if the trees planted artificially in sites with frost before 113°C thermal day. The weakness of the hypothesis is to assume that the thermal time of Sugadaira population of *F. crenata* maintains in the population in Kuromatsunai, Hokkaido, because the thermal time of *F. crenata* population seems to be varying a local by local (Kaji & Takahashi 1999). In order to examine the hypothesis, an additional experiment of transplanting of *Fagus crenata* seedlings grown in Kuromatsunai to the mountain regions is required.

In addition, plant distribution is not determined by only a single factor but various complicated factors affect the distribution. In particular, species to species interaction is important factor to allow the survival of the species, which are even able to adapt to physical environments (Watanabe 1994). The species which are able to pass through a physical threshold must encounter inter species interactions in a plant community.

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要約

ブナ、ミズナラの開葉時期と遅霜に関する実験。小島久子（ライフ計画事務所）、鶴子茂（筑波大学生物科学系）、中村寛（筑波大学農林系）、林一六（筑波大学菅平高原実験センター）

長野県菅平にある筑波大学の樹木園に植栽されたブナとミズナラの開葉時期と、葉の霜に対する耐性について実験を行った。実験にはマイナス5度以下に調節できる生育箱と野で同じような冷却条件が与えられる自然放冷装置を製作して用いた。この自然放冷装置は既報の論文を参考にこの実験のために製作した。同時にブナ群落の分布限界とされている黒松内を中心とした北海道南部各地の温量指数と遅霜出現時期を検討した。開葉時期は1988年から1994年までの7年間記録し、その平均を求めた。それによると、ブナはミズナラより平均10日ほど早く開業し、それに要する日温量数はブナで平均113℃、ミズナラで182℃である。一方、開葉したばかりの葉の霜に対する耐性の実験では、ブナの開業したばかりの葉は霜に遭うと枯死し、開葉前の芽の段階では霜にあっても枯死しなかった。ミズナラは枝の先端に数個の冬芽を付けて、若葉が霜で栄えても側芽が開業し、その後成長できた。それにたいして、ブナの頂芽は前年8月ころから形成され、側芽をもたないので、開業後、遅霜に遭うとその後の成長ができなかった。ミズナラは、開葉時期が遅いことと、側芽を持つことによって、遅霜の害を回避している。開業時期の遅れは、遅霜のない地域では高成長の開始時期の遅れとなり、物質生産においてブナに対して不利である。ブナは光合成を早く開始する代わりに遅霜に遭遇する危険をもつ。この二つの実験から、ブナは日温量指数が113℃日に達した後遅霜がある地域には自然には分布できないが、ミズナラは上に述べた生態的特性によってその地域でも分布できる、より北方に分布を広げることができると思わされる。日温量指数が113℃日に達した後に遅霜がある地域を北海道南部で調べてみると、倉見安と岩見沢が相当する。ブナが分布できる黒松内と倉見安のあいだには羊蹄山、ニセコアンヌプリなどの山塊があり、この山塊付近が113℃C日に達した後に遅霜がある地域に当たりブナの自然分布を妨げていると考えられる。これをブナの北限を説明する開業時期-遅霜仮説とする。この仮説から、日温量指数が113℃日に達する前に最後の遅霜のある地域で黒松内以北でもブナは生育できるので、人為的に植えればブナは生育できるであろう。