Studies in Frost-hardiness of the Japanese and the Dahurian Larch and Their Hybrids

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Preface

The Japanese larch (*Larix leptolepis* GORD.) is one of the quickest-growing forest tree species in Hokkaido, and therefore occupies quite an important position in Hokkaido forestry. This island, especially the part to the northeast of the Ishikari Lowland, lies phytogeographically in the boreal coniferous zone, and its winter climate is much severer than that in the natural distribution area of this species. Young nursery stocks of this larch are injured by early frosts, and sometimes by late frosts, also, more frequently in Hokkaido than in its homeland (SUGIMOTO '61). The dahurian larch (*L. gmelinii* GORD.)**, on the contray, shows sufficient hardiness even in the altitudes of the central mountain areas, for example, in a trial plot at Hakuyo-daira in the Mt. Daisetsu area, which was prepared by TAKAHASHI and HAMAYA in 1963. Thus the hybrids between these two species were expected to be appreciably hardier than the Japanese larch. And in fact young

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** In the present paper, "the dahurian larch" is used to mean var. *gmelinii* in the sense of OSTENFELD & SYRACH LARSEN ('30), and two geographical varieties are excluded from this grouping, i. e., the North-Chinese larch (var. *Principis-Rupprechtii* OSTF. & LARS.) and the Korean larch (var. *olgensis* OSTF. & LARS.).

hybrid saplings at Hakuyo-daira seemed to show a good resistance against early frosts (in middle August and early September) in 1964 and '66.

The hybrids also have a much higher resistance to gnawing by red-backed voles and hares than the Japanese larch. These two advantages may well allow these hybrids to play an important part in the present and future forestry in Hokkaido. But little is known of the mechanism and seasonal changes of frost-hardiness of the larch in general (SAKAI '65) (TAKATOI & WATANABE '61), especially from a stand-point of tree-breeding.

In the autumn of 1965, we had a good fortune to obtain a moderate number of 3-year-old (to be exact, in the 3rd year of growth) seedlings of both species and their hybrids. A series of studies composed of the phenological recording at the nursery and of serial freezing experiments and histological observations in the laboratories were initiated, in order to compare the seasonal process of increase of frost-hardiness thereof and also to find relationships, if any, between the frost-hardiness and the phenological phenomena and the histochemical character. Most of the research work was carried out at Yamabe by KURAHASHI and HAMAYA with the combined assistance of Mr. S. NAKATSUBO, Mr. C. SASAKI, Mr. Y. TAKAHASHI and Mr. T. SHIMA, to whom we are greatly indebted, from August in 1965 till January in 1966. The freezing of the materials was carried out by SAKAI at the Institute of Low Temperature Science, Hokkaido University, in Sapporo. HAMAYA is also responsible for the wording of this article.

Materials

3-year-old seedlings of the following families of the Japanese (L) and the dahurian (G) larch and their hybrids (H) were used in the present study (Tab. 1).

Among the seedlings of each family, at first, 100 individuals were chosen at random to observe and record their phenological characters at predetermined intervals. Another

Table 1. Materials used

Species or combination	Family no.	Mother tree no.	Location of mother trees	No. of seedlings examined
Gs	S-1196	V-95	Y-36	185
Gs	S-1197	V-478	Y-36	200
L×Gk*	S-1206	V-2012×Y-37	Private stand at Yamabe	220
L×Gs*	S-1207	V-2014×Y-36	Ditto	210
Gk×L(?)	S-1201	V-463×(L)	Y-37	265
L	S-1210	V-2012	Private stand at Yamabe	215
L	S-1211	V-2014	Ditto	145

N. B. S-1201 is a progeny of a dahurian larch tree (V-463) surrounded by Japanese larch trees. Among its 2-year-old seedlings, larger ones were selected and regarded as a natural hybrid between them.

Y-36 and -37: No. of seed collecting stands, planted in 1943 and 1934, respectively, in the University Forest.

s: From Saghalien. k: From the S. Kuriles.

*: By artificial pollination with the pollens gathered from several trees and mixed together.

set of random sampling was done on the rest of the family, as the occasion arose, including a part or all of those 100 seedlings after phenological investigations were completed at the beginning of the present study. At intervals of ten or twenty days from September 5th till December 23rd, 5 (10 from S-1201 only) seedlings were subjected to freezing at each grade of temperature each time; and the top pieces of shoots of 3 (5 from S-1201) seedlings were fixed in formalin-acetic acid-alcohol, for the purpose of investigating the seasonal variation of starch content in the parenchyma cells of various tissues. Additional 2~3 seedlings were used as the control each time. For the freezing experiment, the top pieces, 20 cm in length, were taken from the main annual shoots of the seedlings.

Method

1. Freezing experiments.

A. Main experiments.

As mentioned above, the top pieces of 5 (or 10) seedlings were subjected to a grade of temperature in every experiment. The pieces of shoots were excised usually early in the afternoon, and sent posthaste to the Institute of Low Temperature Science in Sapporo by urgent mail at 4 p.m. on every predetermined date, after the needles were all removed.

Before freezing, the pieces of shoots were put in small polyethylene bags after dipping in water to prevent super-cooling. At the Institute, they were first cooled in a chamber at −5℃, and then were gradually transferred at 1-hour intervals to chambers maintained at temperatures graded at 5°-intervals from −5° to −35℃ (in further steps, to −50℃ directly from −35℃, and to −70℃ after cooling at −50℃ for 13 hours). After the pieces were cooled down to the desired temperature, they were held at this temperature for 16 hours (at −70℃ for 3 hours), and then slowly thawed in air at 0℃.

They were sent back to Yamabe by urgent mail again, arriving there usually about 9 a.m., and were at once planted in water in a green house. The water was replaced by cold well water once a day. The green house was heated at about 15℃ (10°~20°) after October 25th. The pieces of shoots under cultivation in water were shaded with reed blinds during the entire period to avoid overheating by direct exposure to the sun. No illumination was used in the green house in the evenings or at night. The control pieces were also excised at the same time and stored in a refrigerator at about 0℃ till the return of the frozen materials.

The dates when the pieces of shoots were to be excised were set on the 5th, 15th (except November and December) and 25th of every month in advance. They will be hereinafter numbered the 1st (Sept. 5th), 2nd (Sept. 15th) and on up to 10th (Dec. 23rd) dates, respectively. Because of the limited number of seedlings, temperatures at which the pieces were frozen were decided and limited to 1~4 grades in consideration of the results obtained from the experiments on the preceding date or dates and in anticipation of a rise in frost-hardiness.

B. Subsidiary experiments.

Subsidiary to the experiments mentioned above, the following were also carried out.

a) 4-hour freezing at −5℃: Some sets of materials were frozen at −5℃ for 4 hours on the 2nd, 3rd and 5th dates, to compare the hardiness in this length of time with that for 16 hours.

b) Hardening at 0℃: On the 4th and 5th dates, some materials were hardened at 0℃ for 2 weeks, to clarify the effect of hardening.

c) Investigation on the hardiness of 2nd pieces of shoots: On the 9th and last dates, second pieces (20 cm long) contiguous to the top pieces were also subjected to the same series of experiments. And a comparison was made between these two parts.

d) Investigation on the hardiness of 9-year-old saplings: It was expected and often actually observed that the frost-hardiness changed (usually increased) with the age of seedlings or saplings. To ascertain this in the larch species, 3 families of 9-year-old saplings were chosen as follows; G: S-303 (seeds from a planted stand at Numakawa, Wakkanai), $G_k \times L$: S-306 (from Y-37, common origin with S-1201) and L: S-311 (seeds from a natural stand at Iwanumata, Pref. Nagano). 10 saplings were chosen at random from each family, and one top piece (20 cm long) of annual shoot from every sapling was subjected to freezing at each grade of temperature each time. The pieces were taken from 2-year-old branches at the top of crowns. The freezing was carried out in the same manner of treatment as above-mentioned on the 9th and last dates.

2. Phenological observations and measurements.

Among several features of the larch species which have been under study by us for the past several years, the 3 following characters seem to have some relationship to the frost-hardiness in autumn, i. e., the time (average) of formation of terminal buds (to be referred to as b-character hereinafter), the time of full suberization of main annual shoots (s) and the time of yellow-colouring of needles (c). All these characters are undeniably subject to the influence of fluctuation of the atmospheric temperatures before and during the period of research. It is also known that these characters are influenced by the age and the growth ability of seedlings.

For later reference, here, the data on temperature (in autumn of 1965) obtained from the screen standing in the corner of the nursery at Yamabe, and the average height and annual increment of height growth of seedlings in each family are shown in Figs. 1 and 2, respectively. In Fig. 1, the uppermost line graph represents the process of decrease of the average of daily maxima for every period of 5 days, the second represents that of temperatures at the regular time (9 a.m.), and the third shows that of the daily minima. And the graph of broken line represents the lowest of minima during the same periods. Small rods are marked on the dates of frost before the beginning of November. Additional information of this place is as follows; Lat.: 43°13′N; Long.: 142°23′E; Alt.: 224 m. Date of first autumn frost in the years of 1946～1965 (average): Oct. 10; the earliest record: Sept. 28. Yearly mean temperature: 7.3℃.

Every five days, 100 sampled seedlings were measured for their height and the

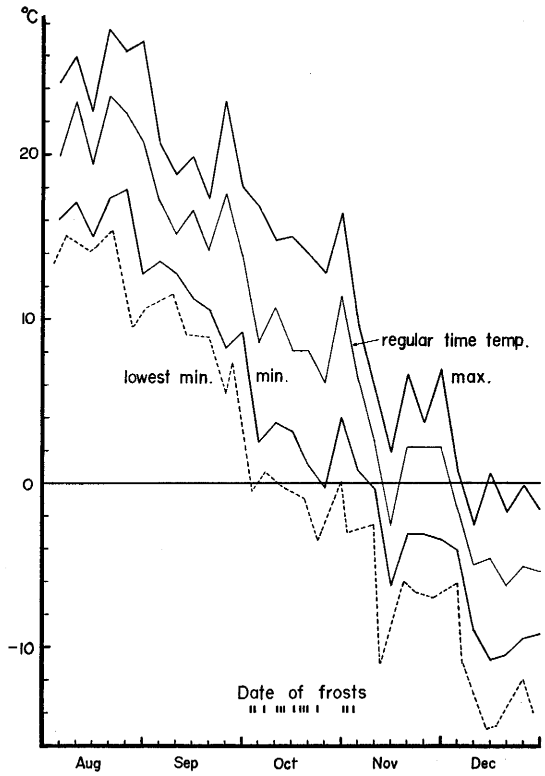

Fig. 1. Process of decrease of atmospheric temperatures at Yamabe in the autumn of 1965.

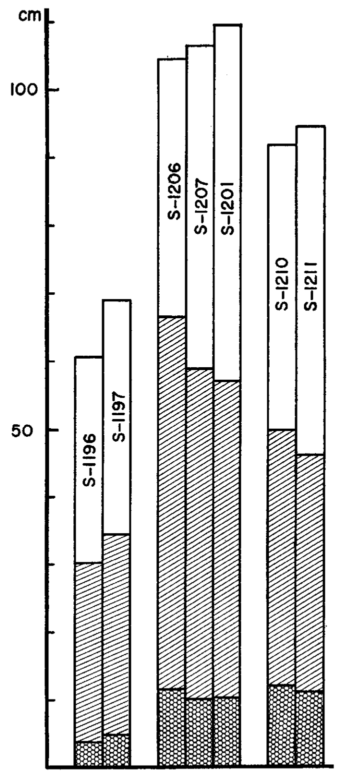

Fig. 2. Average seedling height at the end of the 1st, 2nd and 3rd vegetative period.

length of suberized parts of their main annual shoots. On the same days, individuals which had formed terminal buds visible to the investigators' unaided eyes were recorded. Recordings were also made on the yellow-coloured individuals. As a matter of predetermination, a seedling was considered a yellow-coloured one when nearly all of the needles below the middle (heightwise) of it had turned yellow. The regular investigators continued measurements or observations on seedlings in their charge throughout the entire period of investigation to ensure a uniformity.

3. Study in the change of starch content.

On the same date as the freezing experiment, a piece of shoot was taken from each of the 3 seedlings in every family, and fixed in FAA. Several sections (by hand) from both parts beneath and 5 cm below the terminal bud were stained with iodine-potassium iodide aq., and the starch content of 5 different tissues or tissue systems, i. e., pith, medullary sheath, xylem, phloem (including the cambium and meristematic layers close to the cambium) and cortex (or outer bark), was examined and expressed with a series of relative values, viz. 0 (none), 1, 2, 3 and 4 (largest content). Each total number (0~12) of 3 individuals was regarded as the relative density of starch grains in a tissue of a family at a time.

Results

1. The process of frost-hardening.

A. Determination of the degree of frost-hardiness.

Different criteria were applied to the frost-hardiness in two periods.

a) 1st period: September 5th~October 25th.

5 days after planted in water, cross and longitudinal sections of the pieces of shoots were examined with the unaided eyes or a hand glass concerning the degree of injury by freezing. The parts injured changed in colour from greenish (bark and the inside of buds) or cream-white (wood) colour into brown on the surface of sections. The shade of this brown colour, the obvious decay or destruction of tissues and other minor signs or symptoms were collectively used as the criteria of the injury, as shown in Tab. 2.

Table 2. Expression of injury

Degree of hardiness*	Initial expression of injury	
	Marks	Remarks
2	−	No injury, normal.
1	±	Slightly discoloured, but not so easy to discriminate from the normal.
	+	Discoloured, though not brown, and easily discriminable from the normal.
0	++	Obviously brown.
	+++	Dark brown and destroyed already.

* : Converted from the initial expression of injury and simplified.

According to GLERUM & FARRAR ('66), frost rings were often formed in the xylem of seedlings of the tamarack (*Larix laricina*) and other conifers which showed no external evidence of frost injury or only some needles injured visibly. The materials indicated by the marks (−) and (±) in this table may also have been injured to a considerable degree if observed under the microscope.

As a result of comparison between the part just beneath and those of 5 and 10 cm apart from the terminal bud, it was found that the former was much different from the latter two in the hardiness especially at the early stage of acquisition of hardiness while those of the latter were very similar to each other. The difference of hardiness by parts was also demonstrated by the observations in another nursery where 2-year-old seedlings were severely injured by an early frost at the beginning of October, 1965 (Tab. 3, Pl.). In this case, seedlings whose shoots were injured down to 5 cm below were observed in rather high proportion, while those injured to 10 cm below were scarce. This deviation from the result mentioned above is perhaps due to the difference of age of the seedlings. The seedlings in this nursery were one year younger than those used in the present study and therefore had not become well hardy even at the lower part of their main shoots at the time of the first heavy frost. It is therefore reasonable to express the degree of hardiness of the whole seedling (h) by the following formula:

Table 3. Extent of frost injury in annual shoots of 2-year-old Japanese larch seedlings

Height class of seedlings (cm)	Numbers of seedlings injured								Number of hardy seedlings	
	Top only		To 5 cm below		To 10 cm below		Sum			
~17	2	(33)	2	(33)			4	(67)	2	(33)
~27	14	(54)	2	(8)	2	(8)	18	(69)	8	(31)
~37	16	(31)	10	(19)	2	(4)	28	(54)	24	(46)
~47	8	(40)	4	(20)			12	(60)	8	(40)
~57	2	(50)					2	(50)	2	(50)
Total	42	(38)	18	(17)	4	(4)	64	(59)	44	(41)

Value in parenthesis: percentage to each height class.

$$h=d_1\text{(beneath the terminal bud)}+k\cdot d_2\text{(5 cm below it)}.$$

Where "k" is a constant to be decided under the consideration of physiological or silvicultural significance of frost damage. In the larch species, usually one of the lateral branches or branchlets comes to assume an erect posture and to act as a substitute of the main shoot, when this dies back. On the other hand, it is clear from the observations of the above-mentioned example of frost injury in the nursery (Tab. 3) that the tops of the lateral branches or branchlets were less hardy than the tops of the main shoots. When a considerable length of the main shoots is injured by frosts, therefore, it may possibly mean a fatal blow to some individuals. From a silvicultural point of view, the hardiness of the part 5 cm below the top is thus considered as quite important. As the actual evaluation of "k" itself is very difficult, $k=1$ was simply adopted in the present study. "h" was classified by actual cases as follows:

d_1	2	1	0	1	0	0
d_2	2	2	2	1	1	0
h	4	3	2	2	1	0

A total degree of hardiness of a family in an experiment is shown with $\sum_{n=5} h$ (in S-1201, $\frac{1}{2}\sum_{n=10} h$), varying between 0 and 20. Hereinafter the part beneath the terminal bud will be expressed simply as the top part while that 5 cm below will be expressed as the lower part.

b) 2nd period: after November 5th.

Before this date, it was comparatively easy to judge the degree of injury of the frozen pieces of shoots by macroscopic means within a short time, as mentioned above. In November and December, however, the annual shoots were almost completely suberized, and it became very difficult to judge the hardiness in the same way as that in September and October. The opening of buds was added as another good criterion instead, as used formerly by SAKAI ('64, '66). To be exact, it is desirable to apply this to the first period, likewise. Some of the control pieces of shoots planted in water, however, withered or decayed after 30~40 days or longer without opening of buds, because their buds had not become dormant yet.

As will be fully mentioned later, the control pieces of shoots, which were put under cultivation in water about 20 or 30 days after the average time of formation of terminal buds in each family, could bring the earliest one of the buds on them into opening about 100 (80~120) days after (see Fig. 9). It was around Sept. 15th in G families, while Oct. 15th in L families. During such a long cultivation in water, some of the pieces withered or decayed, and it became nearly impossible for them to perform their rôle as the control. November 5th was thus the earliest day on which this character could be likewise applied to the frozen materials of all families without risk. For L and some of H families, however, about 80 days were still required, and in the G families there were found decays (by fungi ?) with a rather larger frequency than in the 1st period. The macroscopic characters, likewise, were therefore examined as in the 1st period and used together with the opening of buds as the criteria.

When most of the pieces of shoots brought at least one of their buds into opening even in the latest family, all pieces in one experiment were cut up and examined for injuries in the inner part of all buds, and that of the wood at the lower part. Even if the unopened buds appeared not to be injured and were normal externally, some of them sustained injury and discolouring at the lower part of the inside (usually inside the procambium) showing brown spots or dots. These spots can not be regarded as a result of fungus attack, because they were scarcely ever found in the control. The bark, or more strictly speaking, the outer part from the cambium, was hardy at every part on the pieces, and consequently it was excluded from the criteria. The degree of hardiness of each piece was expressed as follows:

1) For buds.

2: Buds opened normally, or all buds were not injured inside.

1: Buds opened abnormally, or at least one or a few buds were injured inside.

0: Buds already decayed and turned dark brown, without opening.

Needles grown from the buds which opened abnormally were curly and very irregular in size or often were collapsed at the tips.

2) For wood.

2: No injury and normal.

1: Discolouring into light brown.

0: Dark brown.

3) Number of days till the opening of buds.

The time elapsed up to the opening of at least one bud was also recorded for each piece of shoot. That of the earliest one in each family is shown in Tabs. 4 and 6. The lag between the earliest and the latest was usually 5~10 days.

The degree of hardiness of the wood at the lower part is '2' in almost all experiments at temperatures higher than −35℃, and consequently the degree of the whole piece may be substituted by that of buds alone. A total degree of hardiness of a family in one experiment ($\sum_{n=5} h$) varys between 0 and 10 ($\sum_{n=10} h=0\sim20$, in the experiment on 9-year-old saplings).

B. Process of frost-hardening in autumn — Results of the main experiments.

The results of freezing experiments in the 1st period are shown by the histograms in Fig. 3. Those in the 2nd period are also shown in Tab. 4. If assuming that a family, whose total degree of hardiness ($\sum h$) is larger than 10 in an experiment in the 1st period and 5 in the 2nd, is as a whole hardy to freezing at a given grade of temperature, its frost-hardiness increases gradually as shown in Fig. 4.

Two G families became hardy to freezing at −5℃ on the 3rd date (Sept. 25), while L families did so on the 6th (Oct. 25) with a lag of about a month. Among three H famillies, S-1206 (L×Gk) is similar to the former, S-1201 (Gk×L) is close to the latter, and S-1207 (L×Gs) falls between them. Hereinafter, h^{-5}, h^{-10}, and so on, represent the frost-hardiness to −5°, −10℃ and so on and the times of attaining these hardiness, respectively. All seven families acquired the hardiness to freezing at −15℃ (or −20°

Table 4. The results of freezing experiments in the 2nd period (after Nov. 5).

Family	Item	Nov. 5 1)				Nov. 25 2)					Dec. 8 3)				Dec. 23 4)				
		Cont.	−10*	−15	−20	Cont.	−15	−20	−25	−30	Cont.	−20	−25	−30	Cont.	−25	−35	−50	−70
S-1196	t_2	36		27	17	15		15	15	15	12	12	12	27	10		30	35	ε0
	B			10	10			10	6	10		10	10	5			4^{3}	3	4^{2}
	W			10	10			10	10	10		10	10	3^{10}			8	3^{4}	5^{2}
S-1197	t_2	36		17	17	15		15	15	40	17	12	12	32	10		25	30	25
	B			10	10			10	9	5		10	10	5			3^{4}	5^{1}	5^{3}
	W			6	6			10	10	10		10	4^{10}	10			8^{10}	5^{4}	5^{4}
S-1206	t_2	61	81	71		30		40	30	30	32	17	17	32	15		35	35	35
	B		10	10				10	8	5		10	10	5			2^{4}	1^{5}	1^{4}
	W		10	8				10	10	10		10	9^{10}	8^{10}			4^{5}	2^{5}	1^{3}
S-1207	t_2	76	76	76		30	45	35	20		27	27	17	22	10		25	—	35
	B		10	10			10	10	7			10	10	5			2^{4}	0^{3}	2^{1}
	W		10	10			10	10	10			10	9^{10}	10			4^{5}	4	5^{2}
S-1201	t_2	76	31	51		47	45	25	25		27	17	12	32	25	25	—	—	
	B		10	10			10	10	6			10	10	5		10	0^{2}	0^{1}	*0*
	W		10	10			10	10	10			10	8^{10}	10		10	5^{2}	5^{4}	*1*
S-1210	t_2	81	81	17		50	60	40	40		27	32	47	47	25	25	—	35	
	B		10	10			10	10	8			10	5^{10}	5		10	0^{3}	1^{4}	*3*
	W		10	10			10	10	10			10	10	10		10	5^{6}	5^{9}	*5*
S-1211	t_2	76	81	81		55	60	40	40		37	27	22	37					
	B		10	10			10	10	5			10	5^{10}	5					
	W		10	10			10	10	10			10	10	10					

N. B. *: Temperatures of freezing at centigrad.
t_2: Number of days, see page 215.
B: Total degree of hardiness ($\sum d'$) of the buds.
W: Ditto, of the wood.
Small italic letters: Values of *B* and *W* of the 2nd pieces of shoots (see page 210).
1), 2), 3) and 4): Materials were dissected for examination after cultivation in water for 81, 60, 47 and 42 days, respectively.

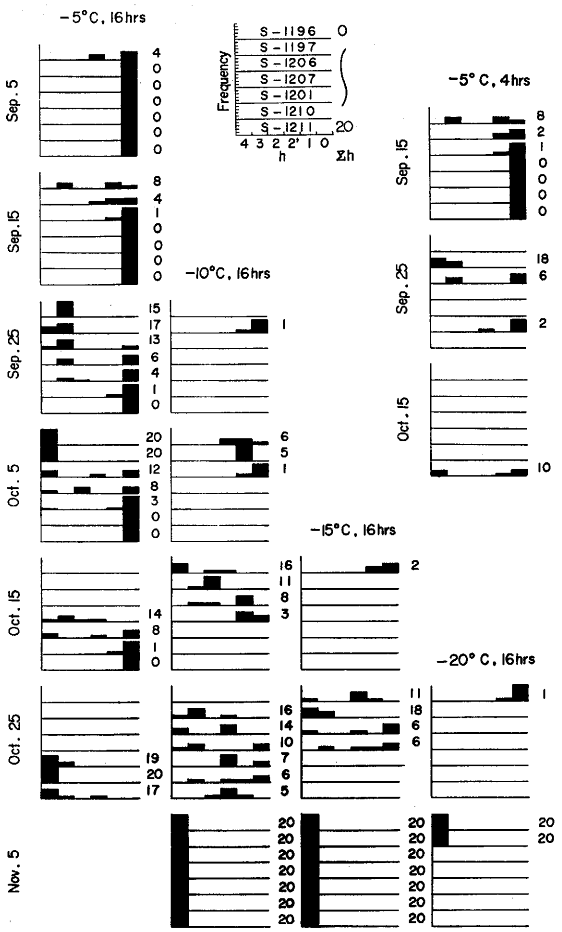

Fig. 3. The results of freezing experiments in the 1st period (before Nov. 5).

in G) almost simultaneously on the 6th or 7th date, and reached their maximum hardiness to −25℃ (−20°∼−30°) on the 8th date (Nov. 25). Therefore it is understood from these facts, that the frost-hardiness in two G and an H (S-1206) families makes a gentle ascent from earlier dates with a temporary slow-down in the course from h^{-5} to h^{-10} while it makes a rapid ascent from later dates with none or slighter stagnation in L and the other H families. The process attaining the hardiness to −10℃ after the stagnation in G and some of H families is probably not so rapid as shown in Fig. 4, but is rather gradual as understood from the graphs in Fig. 5, though the presence of the stagnation itself can not be neglected. The rapidity may have resulted from the assumption itself that families with total degrees of hardiness larger than 10 were regarded as healthy or hardy.

The processes attaining the hardiness to −5℃ are especially different among the families or family groups. Two G families began to acquire h^{-5} as early as the middle of September and increased somewhat gently as shown in the indented graphs, and three H families increaed more gently and indentedly. On the other hand, L families acquired nearly full hardiness at a leap from the minimum at the end of October, keeping up similar rectilinear rapidity at every 5°-interval of temperature.

If similarly assuming that the pieces of shoots, whose degrees of hardiness (h) are larger than 2, are regarded as hardy, there is naturally found an unevenness in the time of attaining that hardiness among 5 pieces (individuals) of a family. The unevenness is, at comparatively slight freezing, especially larger in H families than in G and L families, as shown by the distribution of double circles in Fig. 4 which represent the presence of one or two pieces with sufficiently high hardiness in an experiment concerned.

As mentioned above, the lower part is hardier or becomes hardy earlier than the top part. Fig. 5 shows these circumstances as to the respective representatives of G, H and L families in the experiments of freezing at −5° and −10℃. A case of the largest

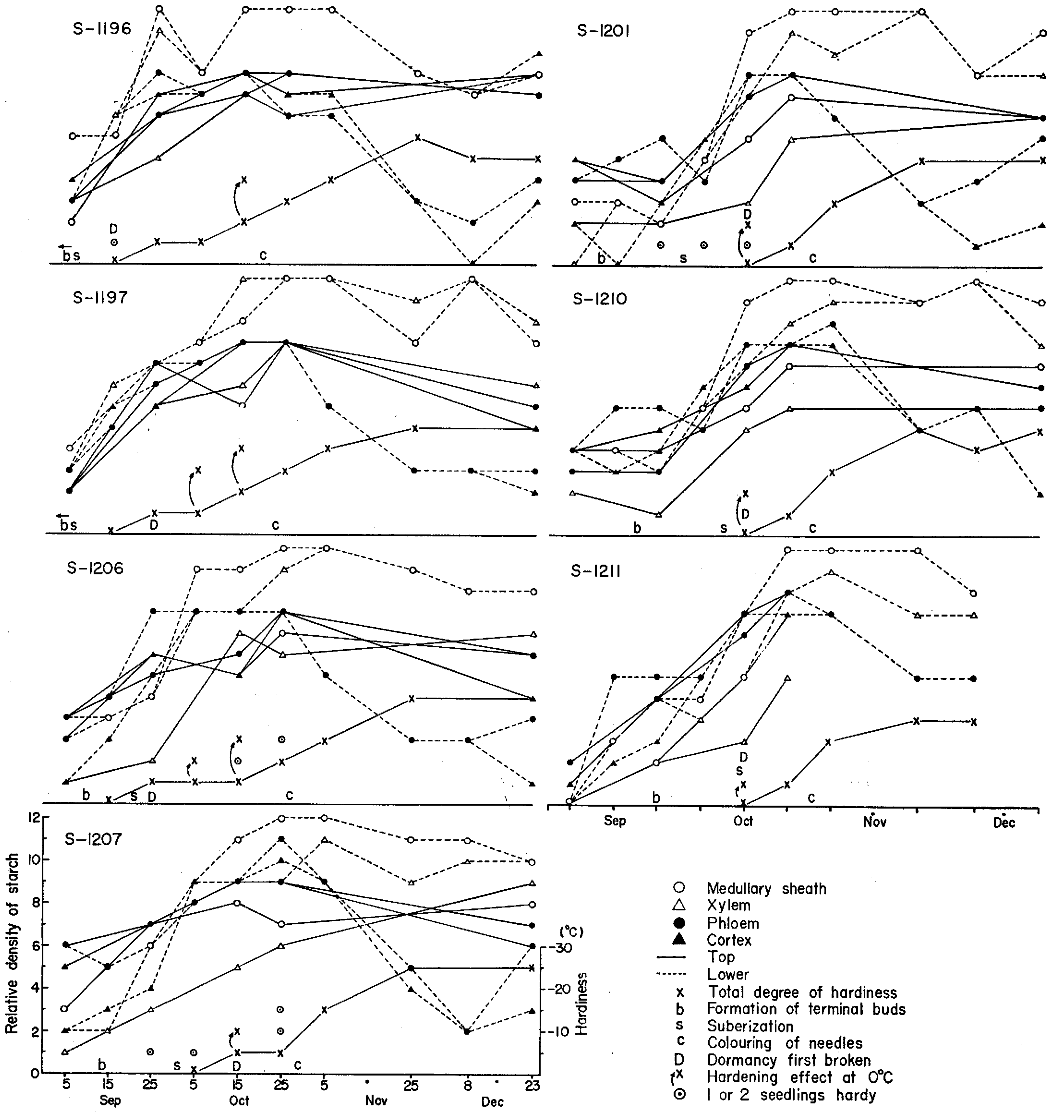

Fig. 4. Process of the increase and decrease of starch content and of the rise of frost-hardiness and the times of occurrence of phenological characters.

lags between the top and the lower parts is found in the experiment on a G family (S-1197) at −10℃ on the 5th date (Oct. 15). In this case, the lower parts had reached the same level of hardiness about 20 days earlier (on the 3rd date) than the top. The top parts of almost all pieces of shoots were most severely injured by that freezing, while the lower parts were completely hardy. And therefore the total degree of hardiness was barely over the standard of '10' mark. An H family (S-1207) shows a contrasting example in the experiment at −10℃ on the 6th date. The total degree of the lower parts in it was 6 and slightly over the standard '5' for the lower parts alone, while that of the top parts was 4 and close to the standard. The sum of these two values was 10 and could not be over the standard of '10'. These two cases may be extraordinary, but the presence of them itself is a good reason for adopting the composite value from both parts as the degree of frost-hardiness of an individual. In other cases, both graphs of the top and the lower part run parallel to each other with rather small distances or lags. The distances between the values of a family gained in different experiments on the same dates, which are marked with asterisks in the figure, seem based mainly on the unevenness of the pieces of shoots or individuals. This parallel relationship between the top and the lower part is likewise explained by Fig. 6 in which the degrees of hardiness of the wood, the bark and the buds in the 1st period are shown. The total degrees of hardiness of these tissue groups or organs ($\sum_{n=5} h = 0 \sim 10$) are usually very close to each other at each part and in every experiment or at times coincide entirely. If remotely compared, however, the wood is only a little hardier than the bark in most cases, with the exception of the top parts of G families and an H family (S-1206), in which the wood is less hardy on the contrary. The buds are the hardiest in every case. And this fact is quite contrary to the results obtained in the 2nd period when the buds are the most susceptible.

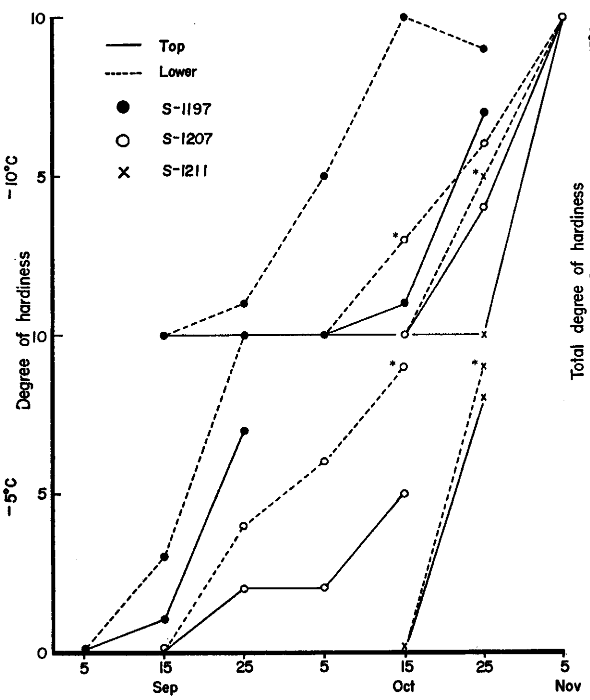

Fig. 5. Process of increase of hardiness in the top and the lower part of shoot in the 1st period (before Nov. 5).

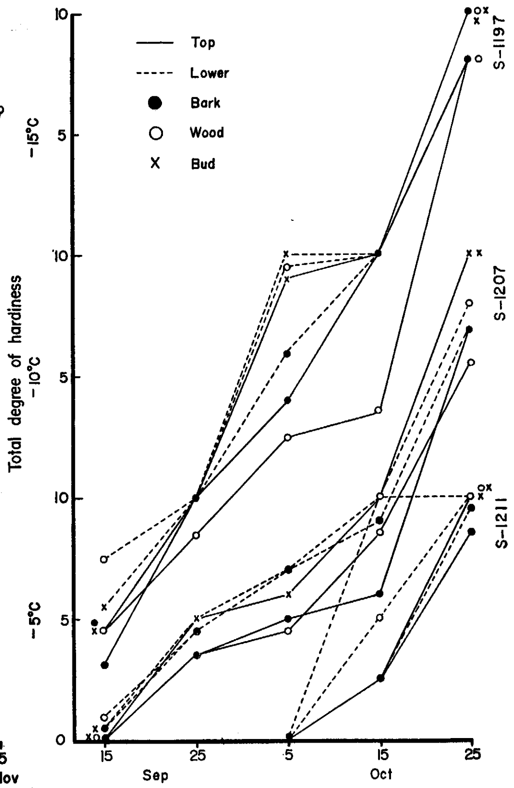

Fig. 6. Process of increase of hardiness in the wood, the bark and the buds in the top and the lower part of shoot in the 1st period (before Nov. 5).

The lowest temperature at which the pieces of shoots survive seems to be −25℃ at the end of December. Though the hardiness to this temperature was not stable in every family on the 8th and 9th dates yet, it became stable even in L and H families on the last date (Tab. 4). The hardiness to −30℃ on the 9th date was naturally not fully attained in all the families. The experiments on the last date had been carried out before the result was obtained from those of the 9th date, and did not include freezing at −30℃. But it seems not likely that the families used in the present study may have shown the total degree constantly beyond the standard mark of '5' in freezing at −30℃. TAKATOI & WATANABE ('62) reported, in fact, that 2-year-old seedlings of the Japanese larch could not survive freezing at −30℃ for 24 hours late in January, 1962. And consequently it is hard to consider that larch seedlings become increasingly hardy in winter after the end of December. On the other hand, 9-year-old saplings could fully survive freezing at that temperature (but not at −35℃) on the 9th date (cf. page 211). It may be thus affirmed that seedlings or young saplings of the larch species can winter at minimum temperatures around −30℃.

C. Results of the subsidiary experiments and their relationship with the mechanism of frost-hardening.

a) 4-hour freezing at −5℃.

The results of this series of experiments are represented by the histograms in Fig. 3. The experiments were carried out on the 2nd, 3rd and 5th dates, and these dates were unexpectedly not many days before or after the first frost on October 3rd. Generally speaking, the total degree of hardiness of each family in these experiments was not so different from that of the freezing for 16 hours, a little hardier in some families and less so in the others. From a technical point of view, 4 hours is perhaps the shortest for freezing the materials in an even condition. And such an extent of cold or frost (−5℃, 4 hours) is actually observable in mornings when early frosts fall. The first frost fell at the nursery on October 3rd, 1965, when the minimum was −0.5℃ in the screen. But it did not cause any injury on the seedlings standing in the nursery. On the other hand, as mentioned above (Tab. 3), the frost in the same morning gave severe damage to 2-year-old seedlings in the other nursery where the minimum was estimated at −3.5°~−4℃ at the height of the screen on the basis of the records in the nearest weather stations. Thus it is possible to mention that damage on seedlings by heavy frosts is very similar to that by artificial freezing at −5℃ (or lower) for 16 hours as well as 4 hours.

b) Effect of hardening at 0℃.

Pieces of shoots were taken from some families on the 4th and 5th (Oct. 5 and 15) dates and hardened at 0℃ for 2 weeks. The results of experiments following the hardening are represented by total degrees of hardiness in Tab. 5. The number of experiments was not satisfactory because of the shortage of materials, but none the less they gave a few interesting points of information on the effect of hardening.

The pieces of shoots hardened effectively could survive freezing at temperatures one

Table 5. The results ($\sum h$) of freezing experiments after the hardening at 0°C for 2 weeks.

Family	Oct. 5			Oct. 15			
	−5*	−10	−15	−5	−10	−15	−20
S-1196	*20*	*6*			*16*	*2*	20
S-1197	*20*	*5* 20	20		*11*	20	20
S-1206	*12*	*1* 18			*8* 18	20	
S-1207	*8*			*14*	*3* 14		
S-1201	*3*			*8* 19	18		
S-1210	*0* 6			*1* 18	12		
S-1211	*0*			*0* 19	6		

N. B. * : Temperatures of freezing at centigrad.
Small italic letters: Total degree of hardiness of the materials immediately subjected to freezing.

or two 5°-intervals lower than those subjected to freezing immediately, as shown with arrows in Fig. 4.

In L families (e. g., S-1210), the total degree of hardiness ($\sum h$) for the experiment at −5°C on the 4th date was 6 and was not beyond the standard of '10', while on the 5th date it was 18 (19 in S-1211) and close to the full mak of '20'. Those of the unhardened were 0 and 1 (0 in S-1211), respectively. Therefore it is concluded that the hardened seedlings of L families become hardy to freezing at −5°C about 10 days or above earlier than those not hardened and show the first touch of hardening effect still earlier. But it is also possible to mention from another angle that crossing points of lines, which are imaginarily drawn horizontally from the dots indicated by arrow marks, with the line graphs in Fig. 4 are located just above the points of about 2 weeks (10~20 days) after the excision of shoots. In other words, if the pieces of shoots were left on the seedlings without excision for 2 weeks, they surely were effectively hardened to the same degree under the climatic conditions of mid-October as in cooling chambers.

That is to say, the hardening of seedlings of the larch species at 0°C in cooling chambers progresses nearly as fast and begins to show its effectiveness as early as under the natural conditions of October. But in November, the effect of hardening at 0°C probably becomes smaller as the surrounding temperature falls (SAKAI '64).

c) The hardiness of the 2nd pieces of shoots.

The 2nd pieces of shoots contiguous to the top pieces were frozen in the same series of experiments as the latter on the 9th and last dates, and examined for hardiness of the buds and the wood of the parts 5 cm below the top ends. The results are shown in Tab. 4, and represented by small italic letters, when differnt from those of the top pieces. The hardiness to every grade of freezing tested on the 9th date was nearly equal to that of the top pieces, with the exception of the buds in L families. At the experiments on the last date, most of the top and the 2nd pieces, except for the wood in some pieces, could not survive freezings at −35°, −50° and −70°C, judging by the criterion of the present study.

It is clear from these results that in December the whole annual shoots are hardy to freezing at temperatures higher than −30℃ (or −25°) in most of the families. But, by freezing at −30°C or lower, the buds are injured so severely that they can open only abnormally, while the wood of some pieces is not or slightly injured.

d) The hardiness of 9-year-old saplings.

As understood from the results of the experiments shown in Tab. 6, 9-year-old saplings of G, L and H families raised their hardiness to −30℃ or higher temperatures up to the full mark both in the buds and the wood on the 9th date. We have seen, on the other hand, that the buds of 3-year-old seedlings were not fully hardy to −30℃ (−25° for L families only). In the experiments at −35°, −50° and −70℃, all sets of pieces were not hardy beyond the standard of '10', with the exception of the wood in G families at −35℃; any large disparity of hardiness could not be found between the seedlings of different ages, neither were the times from the beginning of cultivation in water till the opening of the buds, in the frozen materials as well as in the control.

Table 6. The results of freezing experiments on 9-year-old saplings.

Family		Item	Dec. 8				Dec. 23			
			Cont.	−20*	−25	−30	Cont.	−35	−50	−70
Gs	S-303	t_2	12—17	12—17	12	12	15	30	35	35
		B		20	20	20		4	3	4
		W		20	20	20		16	10	7
Gk×L	S-306	t_2	17—22	17—22	17—22	12—17	20—25	35	35	30
		B		20	20	20		5	6	5
		W		20	20	20		10	5	3
L	S-311	t_2	27—42	22—32	22—37	22—27	30—35	—	35	—
		B		20	20	20		0	1	0
		W		20	20	20		9	4	0

N. B. * : Temperatures of freezing at centigrad.
t_2, B, W: See the notes of Table 4.
All materials were dissected for examination after cultivation in water for 42 days.

As mentioned before, the 2-year-old L seedlings were injured by the first frost early in October, 1965 by which 3-year-old seedlings were not injured; and the pieces of shoots of seedlings of the same age were thoroughly killed by freezing at −30℃ for 24 hours (TAKATOI & WATANABE '62). Therefore a tendency of rising of maximum hardiness with age is in evidence. But the extent of this rise is not so large but only from h^{-25} in the 2 (or 3)-year-old seedlings to $h^{-30-\alpha}$ in the 9-year-old (α: small). The cold lower than −30°C (or −35°) is probably too severe even to older plants of the larch species when annual shoots of them are cut off and frozen at those temperatures in the same way as in the present study.

2. Phenological observations.

A. Phenological characters.

Fig. 7 shows the respective histograms of the b-, the s- and the c-character of

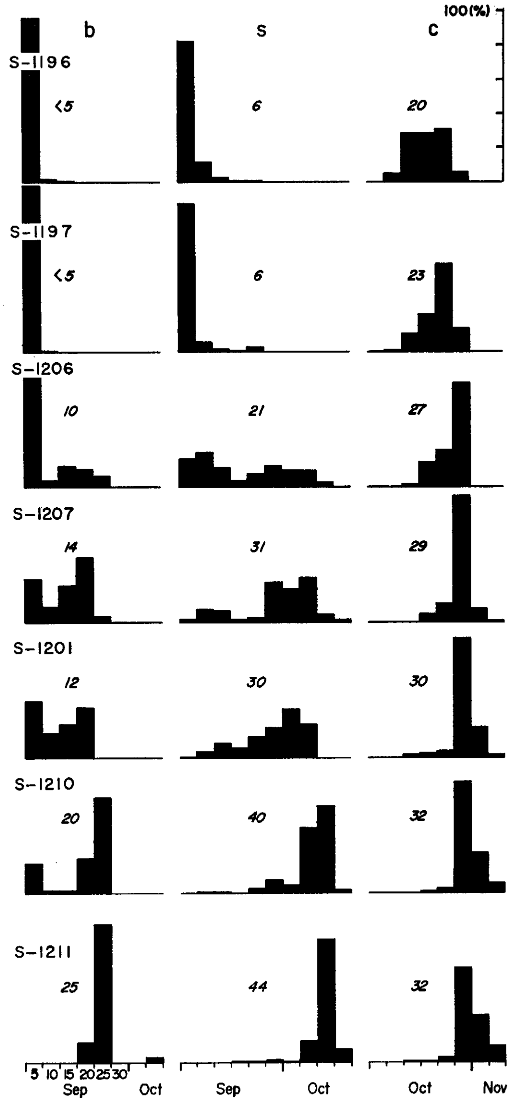

Fig. 7. Variations in the times of terminal bud formation (b), full suberization (s) and colouring of needles (c) for 100 seedlings.

every family. The italic letter accompanying each histogram indicates the average number of days counted from August 31st for the b- and the s-character and from September 30th for the c-character. The last day of August was too late to regard as the starting date of observation on the b- and the s-character, especially for G families. As understood from this figure, L families differ sharply from G families in the b- and the s-character. Three H families are intermediate between them in the averages and show much larger variations (variances). On the other hand, the c-character does not make a clear-cut distinction among the families or family groups with a lag of only 12 days at the most.

When these characters are compared from another point of view, the differences among the families or family groups and their seasonal changes become more obvious. Fig. 8 shows the processes of increase in the length of main annual shoots and in the relative length of the suberized parts of them as averages of 100 seedlings at regular intervals.

The families show similar graphs of increase of length in each species or hybrid. 2 G families ceased to show an increase in length before the first date, while L families did so around the end of September. H families reached the final height on the 20th or 25th of the same month. Similar tendencies are also recognized in the b-character. It is usually observed among the nursery stock and saplings that the main annual shoots grow still a few centimetres in about 10 days after the formation of terminal buds.

The suberization is somewhat later than this. That of G families was completed in the middle of September, while that of H and L families was finished at the beginning and the middle of October, respectively. As seen in Fig. 1, the first frost fell at the beginning of October, when a cold air wave from Manchuria swept over Hokkaido.

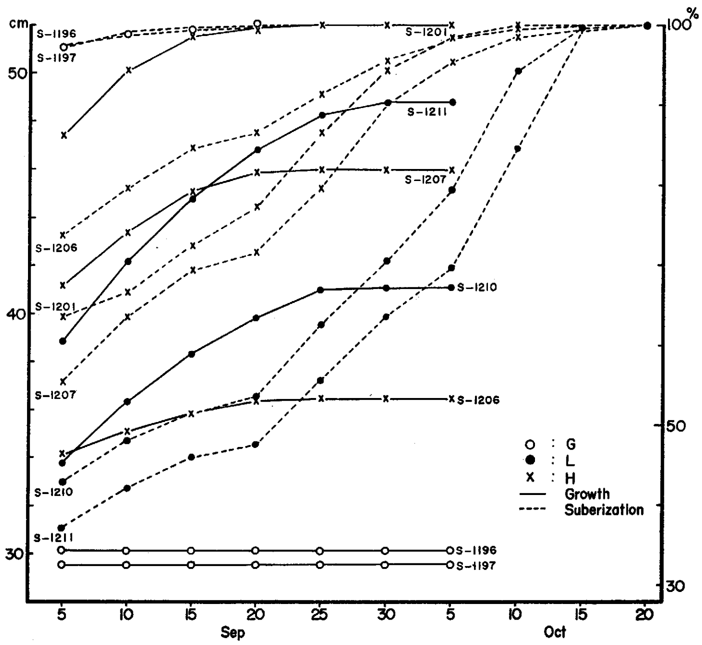

Fig. 8. Processes of increase in the length of main annual shoots and in the relative length of suberized parts of them as averages of 100 seedlings.

At that time some seedlings of L and H families had not completed the suberization of their shoots, and, as a result, a large number of 2-year-old L seedlings, which were doubtlessly less suberized than the 3-year-old seedlings, were severely injured down to the lower part of shoots by the frost, as mentioned above.

Some apparent coincidences can be recognized between these characters and the rise of frost-hardiness, as shown in Figs. 4 and 10. The s-character occurs generally just before the first appearance of frost-hardiness. The discrepancy is inevitably larger in H families (especially in S-1201). The b-character appears 10∼20 days earlier than the s-character and consequently (20∼)30∼40 days earlier than the acquisition of frost-hardiness (h^{-5}), as TAKATOI reported ('60). For G families, as mentioned above, the observation was started too late for the b- and the s-character. The appearance of the c-character is only about 5 (2∼8) days earlier than h^{-15}, and consequently it is possible to consider that the colouring of needles nearly coincides with the gain of h^{-15} and nearly simultaneously in all the families. The process of rise of frost-hardiness after that also is accordingly not so different among the families.

According to the former observations, some of the phenological characters, especially the formation of terminal buds, appeared somewhat earlier as the seedlings became older —the largest difference was usually found between 3- and 2-year-old seedlings (1∼2 weeks in L and G and about a month in *Larix decidua*)—, or when the seedlings of the same age were not transplanted. Therefore it is also surmised that the older or untransplanted seedlings or saplings acquire their hardiness to slight freezing earlier than the younger or the transplanted.

B. Dormancy of the buds.

As briefly mentioned above, the buds on the pieces of shoots can open or can not in accordance with the time of beginning of the cultivation in water. And this character was applied as one of the criteria for judging the frost-hardiness especially in the 2nd period. With special consideration to the dormancy of foliar buds from the very beginning of the present study, careful observations and recordings were made concerning the opening of buds. In Fig. 9, the line graphs show how many days were required for the opening of buds from the beginning of cultivation in water. Other signs represent those of the materials frozen in the experiments, and are plotted between two boundary lines

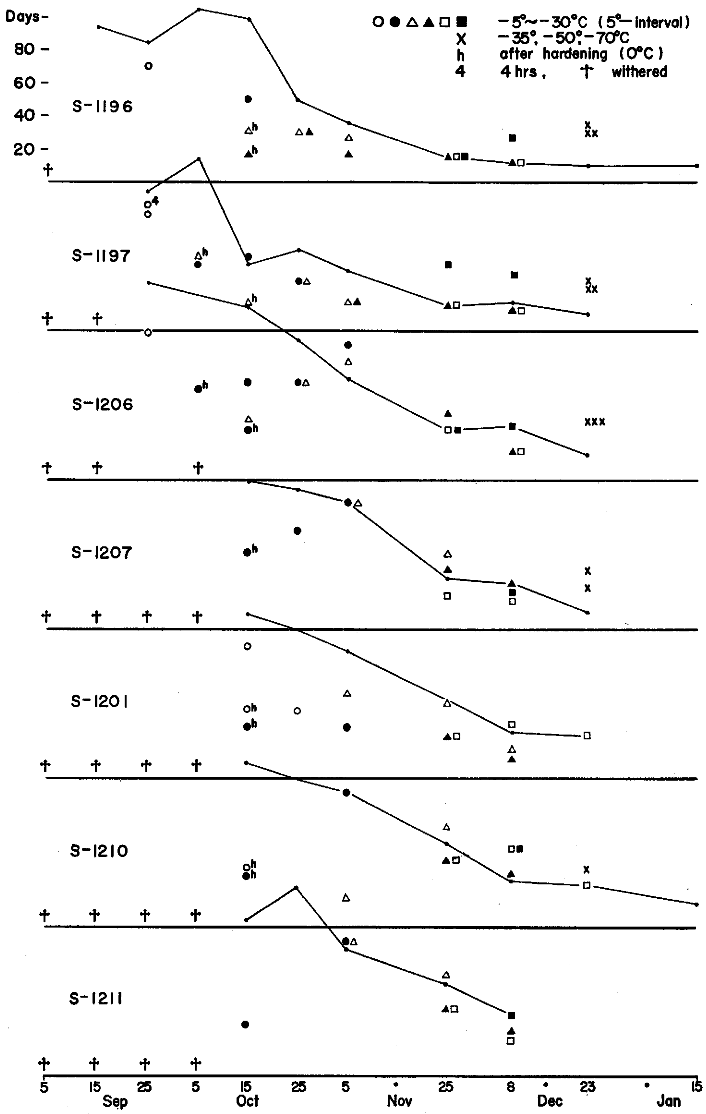

Fig. 9. Times elapsed from the beginning of cultivation in water till the opening of buds.

without exception.

The control pieces of shoots, excised within 15~30 days after the formation of terminal buds, were dead under water cultivation conditions without opening of buds. On the pieces of shoots excised after a longer time, however, the buds came to open after cultivation for about 90~100 (85~115) days. This first possibility of hastening the opening of buds (substituted by 'D' hereinafter) appears in the latter half of September in G families, while in the middle of October in H (except S-1206) and L families. The times (in number of days) required for the buds to open then show similar graphs of decrease among all the families with some inconsiderable irregularities. And after some point of time the buds in each family came to open after cultivation in water for a certain length of time, i. e., about 10~15 days. This may be due to termination of the dormancy of the buds. The time was at the end of November in G families, while being at the end of December or the beginning of January in H and L families. Thus both the first possibility of hastening the opening of buds (D) and the termination of dormancy are about a month earlier in G familes than in L and H families. On the other hand, the length of time elapsed from the former (D) till the latter was about 80 day in every family. In Fig. 9, all the graphs show lines with nearly the same gradient if the lines are regarded as continuous or straight without breaks before the termination of dormancy. If the period from D till the point of time of putting materials under cultivation in water is called t_1, and that till the opening of buds from the beginning of cultivation is called t_2, the following simple equality is given:

$$t_1+t_2=k \text{ (a constant, 90~100 days is this case).}$$

For example, at the end of the dormancy, t_1 is 80 days and t_2 is 10~15 days.

If a comparison is run among Figs. 4, 9 and 10, in every family, D nearly coincides with the first appearance of frost-hardiness to -5°C or is about 10 days earlier. It is also about 0~20 days later than the s-time and about 20~30 days later than the b-time.

Therefore, $k+20(\sim 30)=k'$ (another constant).

This means that the larch species require about 100~110 days to terminate spontaneous dormancy after the formation of terminal buds under natural conditions. And during this period, the control pieces of shoots excised early were kept warm in a green house, while the others were left under outdoor condition of the cold November or December of Hokkaido. Therefore it may be possible to consider that the dormancy, though essential to the opening of buds, is itself not stimulated nor progressed by the conditions of moderate temperatures. In other words, a certain length of time (ca. 100 days) is indispensable for the larch species to terminate the dormancy when they are under the natural condition of temperatures or at higher temperatures in a green house. (Here, it should not be forgotten that all needles on the pieces of shoots were removed and that the control pieces were kept in a refrigerator at 0°C for about 3~4 days till the return of the frozen materials. If the pieces of shoots were kept in a green house immediately after the excision and the needles were left on them, the circumstances may probably have differed to some extent from that mentioned here.)

On the other hand, the buds on the pieces of shoots frozen at various temperatures could open in definitely shorter times than those of the control when the materials were treated before and on the 7th date (Nov. 5) in every family. But in the experiments on this date, the buds on the frozen materials opened later than those of the control in S-1206 (H) and S-1211 (L). In the 2nd period after that, no definite differences of earliness between the control and the frozen were found; and the number of days required (t_2) became rather close to that of the control with the exception of those in the experiments at −30℃ and lower temperatures.

Then a question arises: why do only the buds of the materials frozen before November 5th open generally in shorter times than those of the control? A conceivable answer to this question is that the larches can shorten the resting period of their foliar buds when they are chilled at certain degrees of frost for some cumulate length of time. The seedlings were grown in a nursery bed before the pieces of shoots were cut off from them. The atmospheric temperatures (9 a. m.) and even the average of daily minima in every period of 5 days did not fall below 0℃ in October as seen in Fig. 1. In November, on the contrary, the averages of minima could be above 0℃ no longer, and those of the atmospheric temperatures were also below 2℃ except at the beginning of the month. Though the seedlings must have been chilled at some degrees of frost on the ground surface of the nursery sometime in October (e.g., 3, 7 ?, 12, 19 and 23), its cumulate length of time was perhaps not long and the extent of cooling was not large enough to be effective for shortening the resting period. A presumption that cooling at a few degrees of frost can not be effective for shortening the resting period is supported by the following result of experiments: The pieces of shoots hardened at 0℃ for 2 weeks before the freezing required nearly the same number of days (counting from the beginning of hardening) for the opening of their buds as those frozen immediately or, if counting from the dates of freezing experiments, as those excised on the next date and treated at the same temperatures. It is also another fact that the materials frozen at different degrees of frost did give rise to nearly the same extent of hastening of bud opening. If connecting the points of the experiments at −5℃ or lower temperatures, roughly speaking, the connected lines are nearly parallel to those of the controls before Nov. 5th. In other words, it is hard to consider that there are large differences in the effect of shortening the resting period among various degrees of temperatures lower than −5℃ (cf. HASEGAWA & TSUBOI '60). Therefore the highest of the temperatures at which the cooling can be effective for shortening must be between 0° and −5℃. It is well known (AOKI '55, ISHIDA '58, SAKAI '63, WATANABE '58) that twigs of tree species begin to be frozen around −3℃ (in *Larix leptolepis*, −2.3°∼−3.7℃, TAKATOI '60). The time necessary to complete freezing of the whole piece of shoot varies with the condition of materials. Therefore, the freezing itself (especially that on the most sensitive part of the shoot, i. e., buds) for a certain length of time is probably the most important factor to shorten the dormant period. So long as it is lower than this, the degree of temperature may not be essential. After November 5th, the seedlings to be used for later experiments had been doubtlessly cooled effectively at low atmospheric temperatures in the nursery; and consequently there was not any definite difference between the control and the materials frozen.

This effect seems to be somewhat similar to that of the hardening at 0℃ or lower temperatures. But the hardening may be based on the effect of chilling at low temperatures which go down from 0° (or from 10°, SAKAI '60a) to −3° and −5℃ (or −10° in some species, SAKAI '64) as the phenological times of the larches advance. As in *Salix, Populus* and other tree species, the hardening at −3℃ gave L seedlings the largest rise of frost-hardiness among various degrees of low temperatures in the experiments in the first half of November (SAKAI '64, '65b, '66b). The starch in the tissues also seems to be transformed to sugars to their largest extent at −3℃ (SAKAI '64). Thus it is possible to consider that both effects represent themselves on the basis of essentially different mechanisms.

The pieces frozen to −30℃ or lower temperatures were injured to some extent and consequently the opening of their buds became abnormal and was retarded.

3. Increase and decrease of starch content.

Five tissues were examined for the starch content in the parenchymatous or other living cells at two parts of the pieces of shoots, just beneath and 5 cm below the terminal buds, at regular intervals. The sum of the values of 3 individuals is here regarded as the relative density of starch grains of each tissue in a family, ranging from a minimum value of 0 to a maximum value of 12, as shown in Fig. 4. Among five tissues, the pith at the lower part is composed of empty cells as is usually the case with that in the matured parts of many plants. At the top part, on the other hand, pith cells are still full of contents. And, because the other tissues are also on their way to differentiation, there is no clear difference in the relative density of starch grains among them. Therefore it seems not necessary to take the density in the pith into consideration.

Among the graphs showing the change of starch content in the other four tissues, especially at the lower part, the graphs of the medullary sheath are very similar to those of the xylem, while those of the phloem and the cortex are also similar to each other. It is thus reasonable to put all of them together into two tissue groups, i. e., the wood for the former two and the bark for the latter two, and to compare changes of the relative density between these groups. During the period of the present study from the beginning of September till the end of December, an increase stage and a high constant stage can be recognized in every graph. (If the starch content is examined quantatively, its seasonal change may generate a continuous curve showing a peak within the area of the high constant stage called so here.) And two further stages, a decrease and a low constant, are also recognized in each of the graphs of the phloem and the cortex at the lower part.

Admitting the presence of unevenness, as inevitable, to some extent because of the limited number of materials and because of the difficulty of quantative expression of the starch content, this steadily increases itself from the beginning of September, or earlier by families, in all the tissues at both parts. The starch content at the lower part reaches the high constant stage almost simultaneously in both tissue groups or with a little lag in the wood. The time is on the 3rd date (Sept. 25) in G families, while in L families on the 5th date. Among three H families, one (S-1206) is the same as the former in this point, while another (S-1201) is the same as the latter. The rest is intermediate. The relative density thereat is higher in the wood [usually (9~) 10~12] than in the bark [usually 8~9 (~11)]. But in the increase stage, a comparison between the two tissue groups is quite difficult.

The starch content in the bark maintains its high constant level for about 10 to 40 days by different families. It then changes into the decrease stage on the 6th (Oct. 25) or the 7th date. It continues to decrease and finally is lowered to the low constant stage on the 8th date (Nov. 25) simultaneously in all the families. But the relative density is different among the family groups, that is, 2~3 in G, 2~4 in H and 5~6 in L families.

Unlike the bark, the wood maintains its high constant level of starch content till the last date with a small decrease. But it is hard to consider that such a high level of relative density in the wood is maintained for a long time after December. (The change of starch content after the end of December was not examined in the present study because of the shortage of materials.) In *Salix sachalinensis,* it was observed that the wood contained very little or entirely no starch at the beginning of the same month (SAKAI '64, '66b).

The change of starch content at the top part is similar to that of the wood at the lower part. It reaches likewise its own high constant stage nearly at the same time as the wood (Oct. 15~25), and then probably maintains the level till the last date. In this case, there is no large difference between the wood and the bark; but the relative density in the bark is, if remotely compared, a little higher than that in the wood in the increase stage. And this relationship in the increase stage seems to reverse itself on the last date in most of the families.

The starch content in the wood at the lower part and in both tissue groups at the top part shows rather little fluctuation, and does not seem to have any direct relationship with the rise of frost-hardiness and the changes of phenological characters, except at the beginning and the end of its increase stage. The tissues at the top still remain in the stage of primary meristem, and their function as storage organs or its activity must be much smaller than the differential function. The wood at the lower part, on the other hand, contains a far less number of parenchyma cells than does the bark, and therefore can store a far less amount of starch in it. There must be some reasons within themselves to maintain a high relative density of starch grains for such a long time, at least, till some time after December.

On the contrary, the changes of starch content in the bark at the lower part seem to be closely related with the rise of frost-hardiness and the changes of phenological characters. As shown in Fig. 4 and 10, there are recognized four main points of time at which the stages shift. The relationships of these points with other phenomena are as follows:

i. The starch content begins to increase about 5~15 days before the average time of the terminal bud formation. This time shall be expressed by a^{cr} (amylum crescens) hereinafter.

ii. The beginning of the high constant stage is only a little (5~20 days) later than the average time of full suberization of main annual shoots or 0~10 days earlier than the time of the first acquisition of hardiness to −5℃ by families. To be referred to as a^{max} (amylum maximum).

iii. The beginning of the decrease stage coincides well with the time of attaining hardiness to −15℃ and consequently with the average time of colouring of needles, also, in every family. To be referred to as a^{dec} (amylum decrescens).

iv. The beginning of the low constant stage coincides entirely with the time of attaining the maximum hardiness (to −25° or −30℃). And a little higher relative density (5~6) in L families may have some relationship with the fact that their seedlings are somewhat less hardy (hardy to −20°, but not fully to −25℃) at this time. To be referred to as a^{min} (amylum minimum).

Besides the coincidences mentioned above, a^{dec} and a^{min} show approximate or entire simultaneity among all the families. And only a^{cr} or a^{max} does not show simultaneity among the families. In consequence, the difference in the change of starch content among the families or family groups can be expressed as the difference in the duration of the high constant stage only, if the relative density after December is not taken into consideration. This difference is parallel to that in the length of periods from the average times of the terminal bud formation (b) or of the full suberization (s) till that of the colouring of needles (c). Generally speaking, the time lag between G and L families is 20~30 days in both the terminal bud formation and a^{max} (or h^{-5}).

The correlations between the change of starch content and the rise of frost-hardiness or the main phenological characters are understood from the mentioned above. And it is possible to suppose a sequence of cause and effect between the decrease of starch content and any further rise of frost-hardiness, especially after the 7th date (Nov. 5). But even before this, the frost-hardiness rises gradually in spite of the continuance in high constant level of the starch content. It is difficult to explain this rise of frost-hardiness by only such histological changes in the annual shoots as the suberization in progress, because the slender annual shoots, even if fully suberized, can hardly withstand the low temperatures of −5°~−15℃ without being frozen. These phenomena seem to be explained rather by the rise of osmotic concentration (SAKAI '57, '62).

Discussions and Conclusions

1. The process of the frost-hardening of the larches in autumn.

The pieces of shoots excised from 3-year-old seedlings of the dahurian and the Japanese larch families could not withstand 5 degrees of frost before the beginning of September, 1965 at Yamabe. Those of the G families acquired the hardiness to this temperature in the middle of the month, and retained it till the beginning or middle of

October. Then they acquired a hardiness to −15℃ at the end of October. By the end of November they attained their maximum hardiness to −25°∼−30℃. The L families, on the other hand, obtained their hardiness to −5℃ at the middle or end of September about a month later than the G families; and soon attained h^{-15} at the beginning of November, about 10 days later than the G families. And it was nearly at the same time with the G families that they acquired their maximum hardiness of h^{-20} or h^{-25}.

The temperature that both larch species can withstand in the depth of winter does not fall lower than −25°∼−30℃ for 2- or 3-year-old seedlings (TAKATOI '60) and does not or falls slightly lower than −30℃ for older saplings, under natural conditions. Although L seedlings are slightly less hardy than G seedlings in December when young, both of them come to withstand the same degrees of frost between −25° and −30℃ or between −30° and −35℃ with the increase of age; and there is no large difference between them concerning this point. This also means that both species can withstand the winter frost in a wider range of Hokkaido (the recent record of the minima was −29.7℃ on Jan. 8th, 1953 at Yamabe). The only difference between them is that the hardiness of G seedlings rises gradually in 30∼35 days from h^{-5} in the middle of September to h^{-15} through a short stagnation at h^{-5} while that of L seedlings rises steeply to the same hardiness from the middle of October in (10∼) 15 days. Therefore this difference may be interpreted as meaning that both species follow a course of frost-hardening through essentially different routes. Comparing this result with that of TAKATOI & WATANABE ('61), however, it becomes obvious that there is not any significant difference between these two routes. TAKATOI & WATANABE kept some sets of 2-year-old L seedlings under short-day conditions by shading from August 16th till September 17th in 1960 in Sapporo, and observed that these seedlings ceased their height growth and formed terminal buds about 20 days earlier than the control. They also found that those shaded seedlings became hardy to −5℃ likewise 20 days earlier. In this respect, if counting the numbers of days from the terminal bud formation (b) till h^{-5} (α), till h^{-10} (β) and till h^{-15} (γ), respectively, on the basis of the results of TAKATOI & WATANABE and of the present study, (α):(β):(γ) [($\gamma-\alpha$) also in brackets] is 30:50:(60∼)70 [35∼40] days for the shaded L seedlings and 30:40:60 [30] days for the control in Sapporo, 30(∼35):40:45 [15] days for the L families and 25(∼30):50:60 [30∼35] days for the G families at Yamabe. And consequently the graphs (Fig. 4) of the G families may nearly coincide with that of the shaded L seedlings, if the latter are controlled to form terminal buds still 10 days earlier and cultivated under natural conditions of temperatures at Yamabe. It is thus concluded that, whether it is G or L, its seedlings, when becoming hardy to −5℃ earlier, require a longer time to attain h^{-15} at the same place. The difference of ($\gamma-\alpha$) between L seedlings at Yamabe (3-year-old) and those in Sapporo (2-year-old) is probably based on, first of all and after due consideration of difference in age, the difference in the fall of atmospheric temperatures, especially of the minima, in autumn between these two places, though there is little difference of latitutdes.

Among 3 families of hybrids, one ($L\times G_k$) shows a similar process to that of G families; another (Gk×L) shows similarity to L families; and the rest (L×Gs) is intermediate between them. And unevenness of the hardiness among individuals in an experiment is larger, as a matter of course, in a hybrid family than in a family of both species.

The process of hardening after attaining h^{-15} is nearly the same for all of the families at Yamabe.

2. Relationships between the frost-hardiness and the other characters.

With the abbreviations and the symbols used in the preceding chapters, the frost-hardiness and the other related characters or phenomena in the representative families of both species are shown in the order of their occurrence, *en bloc,* in a figure (Fig. 10). Several important coincidences at the times of their occurrence were recognized; some of which were explained above. It is apparent from this figure that the most complete coincidence is that of a^{min} and h^{-25} (or h^{-20}) which takes place simultaneously in both species. Coincidences seen individually in each of them are $h^{-5} \fallingdotseq a^{max} \fallingdotseq$ D (with 5~10 days' lag in L) and $h^{-15} \fallingdotseq a^{dec}$ (5 days' lag in G). Intervals between a^{cr} and D also are small, less than 5 days in G and 15~18 days in L. The c-time usually falls between h^{-10} and h^{-15}; but that of the shaded L seedlings occurred before h^{-5} and 15 days after the b-time in TAKATOI's experiment ('60). KOBAYAKAWA ('44) obtained nearly the same results as those of the present study by his short-day treatment, i.e. ca. 40 days after the b-character. Some of these coincidences suggest presence of close relationships between the rise of frost-hardiness and the changes of some of the characters or phenomena.

As briefly mentioned above, remarkable resemblances can also be recognized among

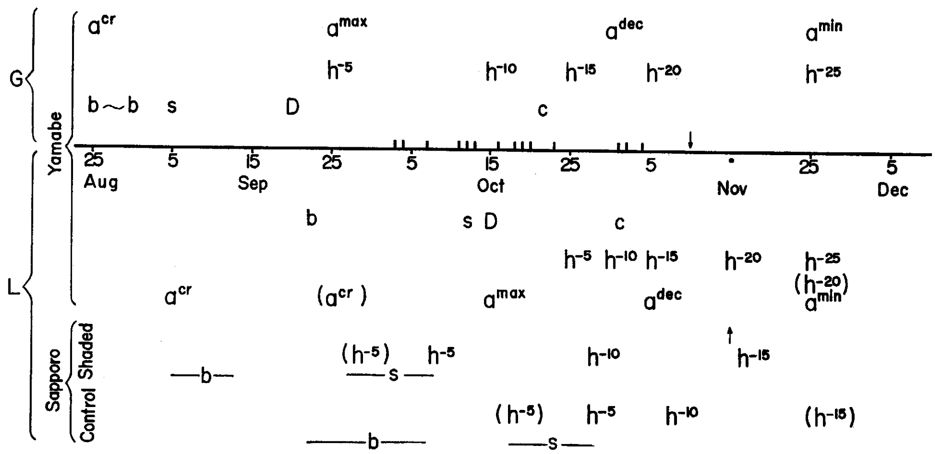

Fig. 10. Relationship between the frost-hardiness and other characters in the times of their occurrence.

N. B.: The data from Sapporo are referred to TAKATOI (1960) and TAKATOI & WATANABE (1961).
Arrows show the dates when the daily minima went down to about 0°C at Yamabe and in Sapporo.
Small rods show the dates of frost before the beginning of November, 1965.
Other abbreviations and signs are shown in the text.

the times elapsed from the b-character till the occurrences of some phenomena or from one stage of starch content to the next, as shown in Tab. 7.

Table 7. Approximate numbers of days elapsed.

	G Yamabe	L Yamabe	L, Sapporo* Control	L, Sapporo* Shaded	H Yamabe
$b-D$	20~25	23			30 (20~40)
$b-s$	5~10 (?)	20	23 (20~30)	23 (20~30)	15~18
$b-h^{-5}$	25~30	33	25~30	25~30	20, 30, 40
$b-h^{-10}$	50	38	38	48	45~50
$a-a$	30 (25~35)	20 (~40)			30 (10~40)

* *apud* TAKATOI & WATANABE ('61)

These two kinds of coincidences or resemblances are of great importance in discussing the mechanism of frost-hardening or frost-resistance. Here it also will be worth while to mention the following. As mentioned above, some of the phenological phenomena occur earlier with the age of seedlings. In Fig. 10, if the respective occurrences of the b-, the s-character, h^{-5} and h^{-10} of the control in Sapporo are advanced by 7~8 days, these coincide nearly with those of the L families at Yamabe, as inferred from the values in Tab. 7, likewise. This means that TAKATOI & WATANABE may have obtained nearly the same results as those of the present study if they had used 3-year-old seedlings as the control insted of the 2-year-old. When the terminal bud formation is hastened still further with the age of seedlings, however, the intervals may become close to those of the shaded L seedlings or the G families under the same condition of temperatures.

In the three hybrids, also, tendencies similar to the above-mentioned are recognized.

3. Mechanism of frost-hardening or resistance.

It has become clear in the preceding paragraphs that changes or occurrences of some characters have close relationship to the rise of frost-hardiness of larch seedlings. In order to explain the mechanism of frost-resistance, sveral kinds of characters or phenomena have been studied concerning their relationships to it in various plant species by a number of specialists (SAKAI '60a). And parallel correlations have been recognized between some of them and the process of frost-hardening. First of all, that between the sugar content and the frost-hardiness was reported by MÜLLER-THURGAV as early as 1886 (PARKER '63); but the opinion is still divided as to whether the change of sugar content is the main factor, or merely one of the main factors, which influences the frost-hardiness, or is simply a result following the cooling at low temperatures and accompanying the rise and fall of frost-hardiness (SAKAI '60a). Correlations are also found between the rise of frost-hardiness in autumn and the decrease of water content in twigs of mulberry (SAKAI '55a, b, '57) and in twigs of the Japanese larch (TAKATOI '60) after the cessation of annual growth, the increase of permeability to water, osmotic concentration and dehydration resistance of parenchyma cells of mulberry and other species

(SAKAI '55b, '59b), the increase of concentration of water soluble protein (SAKAI '57, '58), or the decrease of starch content of *Fatsia* (JIMBO '36) and other species. Among these, the increase of sugar content is closely proportional to that of osmotic concentration and nearly so to that of dehydration resistance, and consequently it is regarded as a factor most directly and closely related to the change of frost-hardiness. The effect of passing through low temperatures in autumn is also evidenced by the fact that the hardiness is raised by artificial chilling or hardening at 0℃ or lower temperatures after a certain stage of annual growth has been attained (SAKAI '56). In this case, also, the effect of hardening seems explainable by the increase of sugar (especially saccharose) content (KOMA *et al.* '55) (SAKAI '59b), which is related to the decrease of starch content to some extent (SAKAI '57).

As it is not the main subject of this paper to discuss fully the fundamental problems on the mechanism of frost-resistance from physiological view-points, however, the process and mechanism of it is sketched here by referring to the literature (KOMA *et al.* '55) (PARKER '63) (SAKAI 55a～'65a) (TERUMOTO '58), as follows:

i) Individuals of plant species which are usually hardy in winter do not become hardy to temperatures lower than the freezing points of themselves until they pass through a certain stage of growth in autumn. At this stage, the cambial activity begins to decline; and the parenchyma cells begin to be filled with starch grains and to increase the concentration of water soluble proteins. On the other hand, the water content in them begins to decrease; and the sugar content may be at its real minimum.

ii) After this, the hardiness gradually rises through the low atmospheric temperatures outdoors in autumn, or, with the same effect, by artificial hardening at 0℃ and then most effectively at about −3℃, and attains the highest degree of hardiness in the depth of winter. Before this stage of growth artificial hardening does not have its effect.

iii) The osmotic concentration varies in parallel with the rise and fall of frost-hardiness from autumn till spring. And the sugar content appears to be the prime factor influencing it, and raises itself by artificial hardening. The starch content shows opposite changes to those of the sugar content, and the transformation of starch into sugar is brought on by chilling at low temperatures or hardening as well. The decrease of water content before and at the stage of growth mentioned above also plays an important rôle in raising the osmotic concentration in spite of low sugar content at that time.

iv) After the osmotic concentration and the sugar content have reached their maxima early in winter, any further rise of frost-hardiness must be attributed to some unknown factors or states of cells.

The above outline will make it easier to explain the mechanism of frost-resistance in larch seedlings from the results of the present study. As explained in the chapter of phenological characters, seedlings, for example, of the G families formed terminal buds late in August. At the same time or a little earlier, starch grains also begin to show themselves under the microscope. When the annual shoots are fully suberized and the relative density of starch grains in their cells is close to its high constant level, the cambium probably shows the fewest cell layers and the water content also falls off close to the beginning of its lowest level (TAKATOI & WATANABE '61), as in mulberry (SAKAI '57). DIETRICHSON ('62) mentioned that differences of frost-hardiness among the races of scots pine (*Pinus sylvestris*), larch species and other conifers in autumn are closely correlated with the termination of growth of the wood which is determined by the histological and histochemical appearance of the cambium and the late wood. The larch seedlings become hardy to temperatures slightly lower than their freezing point by that time, that is, about 20～25 days after the formation of terminal buds. While the sugar (saccharose) content in the bark of twigs of mulberry stays at its minimum for about a month after it, the osmotic concentration continues rising (SAKAI '57). In the larch seedlings, the starch in the bark maintains a high constant level of relative density for a comparatively long period. During this period, the assimilation products being stored in needles or, with a smaller possibility, being the construction matter of needles till then, likewise, must be translocated into the tissues of shoots (HARADA '64) in the form of sugars. And the possible rise of sugar content based on this translocation and the decrease of water content seem to cause the rise of frost-hardiness in the early stage, as will be mentioned later. In *Salix sachalinensis* (SAKAI '64), the osmotic value in the bark likewise begins to rise at the beginning of October; and after that the starch content in the wood maintains its high level for about 20 days and then begins to decrease. The seedlings of L families began to cease their height growth at the end of September, about a month later than the G families. Similar time lags are also found in the first appearance of starch grains, the full suberization and the first rise of frost-hardiness.

It is accordingly possible to consider that, while the seedlings of L families are still growing and consuming the assimilation products from needles for composing the plant bodies, those of G families which have nearly or entirely accomplished their annual growth are storing the products in twigs and other organs at first in the form of starch grains and then in the form of sugars as they mature. The former probably starts on this process of maturation later but with a larger rapidity than the latter. When the needles cease their photosynthetic work—or finish the translocation of the products to the other organs—and turn yellow at last at the end of October or the beginning of November, the starch content of the bark is at the termination of its high constant level with nearly the same relative densities in all the families; and most of the seedlings become hardy to $-10°$ or $-15°C$ evenly or with a lag of about 10 days. This increase of sugar content in G seedlings is naturally not a process separate from the maintenance of the high constant level of starch content. The sugars as the products of assimilation are expected ordinarily to be transformed into starch, as the growth and the cambial activity slow down, as seen in mulberry (SAKAI '57). But about the time when the starch content reaches its high constant level (at the end of September), the minimum temperatures have fallen to about 5°C. And the temperatures falling day by day may prevent this transformation into starch, or may cause a certain amount of starch grains, which are once transformed from the sugars, to be retransformed into sugars. And consequently, the starch content does not increase beyond the high constant level, while the sugar content may be raised gradually. In L seedlings, such an extent of progress is probably finished within a short time in the latter half of October. Furthermore, in the experiment of TAKATOI & WATANABE ('61), 2-year-old L seedlings gradually became hardy to $-3°$ to -10℃ as the water content decreased from about 80% to 50~55%. Therefore such gradual decreases of water content are also considered to play an important rôle, together with the increase of sugar content assumed above, in raising the osmotic concentration and then the frost-hardiness. The colouring of needles in the same L seedlings started after the water content had reached the lowest level of about 50%. As considered from the above-mentioned points, the processes of the increase of sugar content, of the decrease of water content and of the rise of frost-hardiness as a result of the former two are influenced by the time of cessation of annual growth and the condition of temperature under which the seedlings grow. And the difference between G-type (early cessation of growth and early rise of hardiness) and L-type (late cessation and rise) at the same place, that is, under the same condition of temperatures and of day-length, should be explained by only the above-mentioned processes of frost-hardening. TAKATOI *et al.* ('64) plucked all lateral branchlets and short-branch needles from the entire height or from the upper half of the height of young L saplings (ca. 2.5 m tall) on July 18th or August 17th. They also transplanted 2-year-old L seedlings on October 15th (10 days after the b-time) or on November 18th (simultaneously with the s-character). And they found that the buds on these materials were injured by freezing at $-25°$ and -30℃ on December 16th—though the shoots were hardy—while the buds of the control were not injured. In these cases, the starch to transform into sugar in December is suspected to have not been stored abundantly enough to reach its high constant level by reason of the reduction of needles or the transplanting. The result of TAKATOI's experiment ('60) may make one have some doubt about the above interpretation. The 2-year-old L seedlings being under the short-day condition began to turn yellow only 2 weeks after the formation of terminal buds in Sapporo. These seedlings became hardy to -7℃ 10 days after that, and the hardiness rose more rapidly than the untreated seedlings. It is impossible to regard the atmospheric temperatures as the main cause of this case. On the other hand, according to KOBAYAKAWA ('44), the colouring of needles of the larches began not less than 40 days after the formation of terminal buds, though its time was varied under different conditions of day-lengths. In the same study, furthermore, G seedlings under the condition of shorter light period (10 or 12 hrs.) kept their needles green till the end of autumn, and these needles were not injured by the frosts which injured severely the green needles of the seedlings under the condition of longer light period (16 hrs.).

After the colouring of needles, the starch grains stored mainly in the bark of shoots are the only source of the sugar raising its content, and these are transformed into sugars under the condition of low temperatures in November nearly simultaneously for all the families. But the seedlings of L families may probably lag only a little behind those of G families in the transformation of starch (Fig. 4), and consequently they are slightly less hardy (−20°∼−25℃) than the latter (−25℃ or lower) at the beginning of December.

In order to verify the above assumption, as a matter of course, the sugar content and the osmotic concentration should be actually measured on the larches, also. Here, it is necessary to remember that the frost-hardiness of each family was decided by the total degree of hardiness of both the top part and the lower part in the first period (before Nov. 5) and by that of only the buds in the second period (after that). And the remarkable coincidence of the significant points of time between the rise of frost-hardiness decided in such ways and the change of starch content in the bark at the lower part shows that the high level of starch content in the top part and in the wood at the lower part, even in December, does not necessarily mean a low level of sugar content in them. It is true that these parts (especially the buds in the second period) are to some extent less hardy than the bark, as in mulberry (SAKAI '55b), but this weakness is nearly remedied in every experiment at temperatures higher than −25℃ (or −30°) by the end of December. In them, the sugars, possibly supplied from the bark of the hardened parts of annual shoots, are thought to be stored in considerable concentrations together with the starch grains. And as the cold becomes increasingly intense in the depth of winter after December, the starch grains in the wood, also, may be transformed into sugar.

It is naturally undeniable that factors other than the starch content as well as the sugar content also act on the change of the frost-hardiness (SAKAI '64, '66b). Older saplings (9-year-old in the present study) can withstand freezing at lower temperatures than do younger seedlings, at the beginning of December. They, furthermore, cease their annual growth and form terminal buds considerably earlier than the younger. This difference between the older saplings and the younger seedlings looks very similar to that between the G and the L families. It therefore may be understood as the difference between or among the species and, at the same time, between or among the development stages of individuals of the same species responding in various degrees to different conditions of factors such as the day-length.

4. Relationships between the frost-hardiness and photoperiodism and dormancy in the larch species.

a. Photoperiodic response of the larch species.

Seedlings of the dahurian and the Japanese larch, or probably of all species of this genus, become hardy to temperatures lower than their freezing point—about −3°C—, after passing through a certain stage of growth, i. e. the cessation of height growth and the terminal bud formation, even if this stage comes somewhat early either based on the specific difference or by some photoperiodic treatments (ASADA '58) (KOBAYAKAWA '44) (SAKAI '59c) (SATO *et al.* '51) (TAKATOI & WATANABE '61). It is concluded from the comparison of the result of the present study with those of KOBAYAKAWA ('44) and ASADA ('58), as shown in Fig. 11, that L seedlings cease their annual height growth earlier in higher latitudes than in the lower. Though the Japanese larch seems less responsive to the day-length condition than the dahurian, it seems to hasten the cessation of annual growth to some extent in higher latitudes. In Saghalien (KOBAYAKAWA '44), both species did not differ from each other in hastening the formation of terminal buds under the condition of shorter light periods, the length of which were fixed to 10 or 12 hours during the entire period of conditioning. But the conditioning with 14-hour light period caused G seedlings to cease their growth earlier than the control, while the same treatment caused L seedling to cease their growth later than the control. And under the condition of 16-hour light period L seedlings did not form their terminal buds after all. ASADA *et al.* ('65) exposed the 2-year-old seedlings of several larch species and hybrids to an additional 2 hours of light after the sunset every evening for a month around midsummer, and caused some of them to hasten the formation of terminal buds. TAKATOI & WATANABE ('61) obtained a similar result from 2-year-old L seedlings, on the contrary, by shortening the day-length to 7 hours for 40 days from August 20th till September 30th. Thus, though it seems not easy to obtain conclusions concerning photoperiodic responses of the Japanese larch, it is undeniable that photoperiodic treatment is one of the means effective in hastening the cessation of growth of the larch species, and consequently in hastening their first acquisition of frost-hardiness.

GOTHE ('52, '53), DIMPFLMEIER ('59) and others (e. g. ALBENSKIJ) reported that saplings of the European larch (*Larix decidua* MILL.) and its hybrids with the Japanese survived several winters in higher percentages than the Japanese. The European larch seedlings, when 3 years old, form their terminal buds nearly at the same time as the dahurian at Yamabe (Fig. 11). And both species probably do so almost at the same time in Munich (ca. 48°N) or at Sakaehama, Saghalien (ca. 47°30′), too. Even if assuming that only the Japanese larch is a long-day species — other species seem surely to be short-day plants in

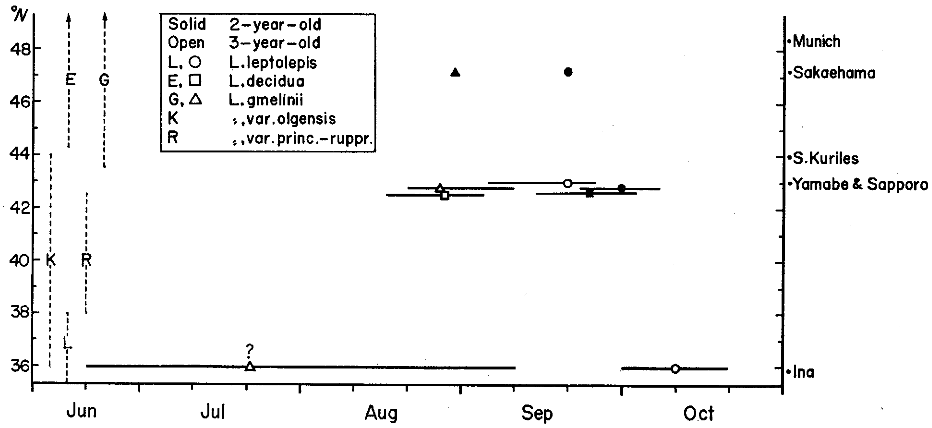

Fig. 11. Times (range and average) of terminal bud formation in various latitudes and latitudinal ranges of distribution of the larch species.
N. B. Sakaehama: Saghalien. Ina: (alt. 760 m) Pref. Nagano, Honshu.

the cessation of growth—, its 3-year-old seedlings possibly form their terminal buds also in Munich at the beginning or middle of September, about 20 days later than the European or the dahurian larch, as seen in Saghalien. (Though the 2-year-old seedlings of the European larch form terminal buds only about a week earlier than those of the Japanese, it occurs with a larger advance of about a month when becoming 3 years old.) Thus early frosts in autumn may have injured only the Japanese larch seedlings or saplings in Munich and other places in Europe. The siberian larch (*L. sibirica* LEDEB.) is also as hardy as the European or the dahurian larch, while the North-Chinese and the Korean or Manchurian larch are as susceptible as the Japanese larch or more.

According to YANAGISAWA ('61b), the groups of L seedlings, which had their origins in higher latitudes within the natural distribution area, ceased their annual growth and turned yellow earlier than those from lower latitudes, at the experiment fields in 3 different parts of Hokkaido. And the damage caused by the frost or drought during the first winter after the planting (in autumn) was smaller in the former groups. A similar difference was found between the groups of seedlings from higher altitudes, with smaller damage, and those from the lower, with larger damage, on the slope of Mt. Fuji. TAMATE ('27) also found that the Japanese larch trees growing at different altitudes defoliated their needles when atmospheric temperatures thereat fell down about 6.5℃ (5.8°～7.2°). The above examples show clearly that, if we wish to choose the best localities (or, if establishable, geographical races) of the Japanese larch for planting in various parts of Hokkaido or to find the most suitable partners with the dahurian larch in breeding hardier hybrids, we have to take the photoperiodic response of them into consideration (YANAGISAWA '61a).

The treatment with naphthaleneacetic acid (TAKATOI '60) or maleic hydrazide solution (SAKAI '59c) can likewise cause the advance of terminal bud formation to L seedlings or mulberry. On the contrary, the treatment with gibberellin solution can prolong the growth period of mulberry and therefore delay the formation of terminal buds and the first acquisition of frost-hardiness (SAKAI '59c). These two groups of chemicals are antagonistic to each other in their actions.

The rising rate of frost-hardiness, after the seedlings have ceased their annual growth, seems to be influenced mainly by the condition of temperature in each locality.

b. Dormancy of the buds in the larch species.

For a while after the formation of terminal buds, larch seedlings probably still retain an ability of opening the new buds in response to some stimulations and of continuing the growth, that is, of forming lammas shoots, as seen in G seedlings under long-day conditions around midsummer (ASADA *et al.* '65) or very often in the Korean larch seedlings. But when the terminal buds turn brown from a green tinge, the latent ability to form lammas shoots seems to be lost. This changing of colour requires about 10 days, according to the observations in the fields. And the shoot tops of seedlings finish their still lasting height growth, probably based on the elongation of cells, almost at the same time. They come to show the first possibility of hastening the opening of buds

(D) soon after this, i.e. about 20～30 days after the terminal bud formation. In this relationship with the bud formation, the dormancy is also closely connected with the frost-hardiness and the photoperiodic response of the larch species; but it seems not unchangeable as understood from the above example of lammas shoots. Even if the terminal buds are formed earlier by the photoperiodic treatment, they do not always become dormant. After opening terminal buds in December and forming new buds in February in the following year in a green house, seedlings of the Korean larch did not open the latter buds in October of the same year yet, while some of L seedlings opened their buds and formed lammas shoots in August or September.

Within a safe limit of no longer forming lammas shoots, however, the terminal buds, formed earlier by short-(or long-)day treatment or by treatment with chemicals, may fall into the resting condition earlier than those of the control, as the buds of G seedlings do earlier than L seedlings. After passing this time of safety, the larch seedlings are appeared to reach the end of their spontaneous resting period in about 90～100 days. This length of the resting period does not seem to be changed so much by the condition of temperatures, either outdoors or in a green house. (Before we enter into further details of these problems, as a matter of course, we should compare the depth of dormancy among nursery stocks from various provenances (ASADA '58) (KOBAYASHI '52) and examine its seasonal variation by continuous observation on abundant materials.)

The earliest sets of shoot pieces of G and L seedlings which could open the buds under cultivation in water were cut off on September 15th and on October 15th, respectively, and kept in a green house after that. The green house was warmed after October 25th in 1965, but the temperatures indoors possibly fell below 7℃ not so many times before then. Even if assuming that the room (and outdoor, also) temperatures were constantly lower than this in the night time from 8 p.m. to 8 a.m. for a month from September 25th till October 25th (see Fig. 1), the sum of hours to which the excised pieces of shoots were exposed to chilling at these temperatures before completing the dormancy, i.e. the so-called chilling requirement (KOBAYASHI '52), is calculated as 360 (12×30) hours at the most. This value is far smaller than those calculated by ASADA ('58), i.e. 552～1008 hrs. with regard to a 32-year-old G tree, and 2328 hrs. with regard to a 5-year-old L sapling. From the above comparison, it is hard to consider that the sum of hours itself is a factor essential to complete dormancy at least for the larch species. The frequency of having been within a certain range of comparatively low temperatures or the stimulation by the chilling at these low temperatures must be more essential. And the sum of hours to which the materials are to be exposed to these temperatures, even when considered as one of essential factors, is probably far shorter than have been considered in the larches and other tree species (KOMA '53) (KOBAYASHI '52). According to ASADA ('58) and ASADA *et al.* ('65), G seedlings form terminal buds within a long period from June to August, and the spontaneous dormancy of the buds finishes in December at Ina in Pref. Nagano, Honshu (somewhat later than at Yamabe). In this case, also, low temperatures not suitable for vegetative activity of the larches seem to come usually later than at Yamabe. The process of completing dormancy may be understood as that of the disappearance of inhibiters of bud opening or that of qualitative and quantitative changes of enzymes or enzyme systems related to this phenomenon (ASADA '58). And the fact that the opening of buds is hastened by the cooling at temperatures lower than their freezing point before the minimum temperatures fall below this, suggests that the disappearance or change, which is usually advanced gradually by passing through a range of low temperatures (e.g. 0°∼7℃) for a certain sum of hours, is possibly given a rapid stride in its progress by the freezing in another range of lower temperatures below about −3℃.

As understood from the above-mentioned details, both the dormancy of buds and the frost-hardiness of the larch species are closely related to the cessation of annual growth under the influence of day-length condition; and when the excised pieces of shoots of larch seedlings become hardy to temperatures lower than their freezing point 20∼30 days after the cessation of growth, they also come to maintain life and to open their buds by the cultivation in water for about 100 days. And therefore it is possible to conceive that both phenomena are bifacial expressions of a single mechanism or at least are under the influence of a common factor during the period from the cessation of growth till the opening of buds. But after an ability to lead the seedlings or the excised pieces of shoots to opening of their buds has been acquired, both phenomena follow different courses and their mechanisms also are suggested to be quite different. Insofar as the present study is concerned, it seems that the so-called spontaneous dormancy (or the rest period in KOBAYASHI's sense, '52) is completed about a month earlier in the dahurian larch (from the end of November to December, cf. ASADA '58) than the Japanese larch (in January). And in the following spring, at Yamabe, the buds of the former open usually from the end of April to the beginning of May only about 5∼10 days earlier than the latter. It follows from this that the resting period following the spontaneous dormancy, i.e. the forced dormancy (or the dormant period in the sense of KOBAYASHI), is not necessarily influenced by the earliness of termination of the spontaneous dormancy, contrary to the supposition of ASADA ('58) and others. According to KURAHASHI *et al.* ('66), the opening of buds of larches and other plant species is closely correlated — rather than with the length of resting period — with the "sum" of daily temperatures (in the day-degree unit) above 0℃ after midwinter; this value is characteristic to each species, at least at each locality. Thus the hardiness of the larch species to late frosts is considered to be under the direct influence of temperatures gradually rising in spring, independent of the spontaneous dormancy.

The above-mentioned is true of the larch hybrids, also.

5. The frost-hardiness and its bearing on tree-breeding and silviculture of the larches and their hybrids.

The process of frost-hardening of the larch species and its mechanism have been explained in detail above. As SAKAI ('59c, '62, '65b) and EGUCHI *et al.* ('66) mentioned, two types of plant species different in the ways of expressing the frost-hardiness are recognized. In one type, closely related species or intraspecific varieties show nearly the same maximum hardiness in the depth of winter, and the difference among them is found in the time when they show the first touch of hardiness, that is, whether the graphs of hardiness are raised earlier or later. In the other, the graphs of hardier species or varieties rising earlier are constantly above those of less hardy varieties, even in the depth of winter. Tea plant (*Thea sinensis*) (SIMURA *apud* SAKAI '59c), roses (SAKAI *apud* SAKAI '59c), and *Cryptomeria* (EGUCHI *et al.* '66) belong to the latter type, while mulberry (*Morus bombycis*) (SAKAI '59c) and poplar varieties (MORITA & SAKAI '66) to the former. And it has been shown by SAKAI ('62, '65b) and others that the larches are also among the former group (though the Japanese larch appears to be a little less hardy in December in the present study). This tendency is not changed even in the cases in which larch seedlings acquire their frost-hardiness earlier under some artificial treatments than natural, as in mulberry (SAKAI '59c). In the present study, furthermore, we have found that the larch hybrids also do not deviate from this generality.

For the practical purpose of comparing the frost-hardiness among the larch species and their hybrids and of selecting hardier groups among them, it is sufficient to consider only how long time their seedlings or saplings, after ceasing the annual growth, can have before early frost falls, insofar as the present knowledges are concerned. The cessation of growth and therefore the frost-hardiness, also, is influenced by day-length conditions or application of such growth hormones or auxin antagonists as mentioned above. In forests or nurseries, however, utilization of the photoperiodic response of the larches is probably the prime, though indirect, way to control their frost-hardiness, under the present condition of practical techniques. This means that, in planting larches in a district where heavy early frosts fall usually, we have to choose among the species, races or families those which can cease their growth in good times under the day-length condition thereat.

If crossings are done between two larch species, races or families which are different from each other in the time of formation of terminal buds, their hybrid progenies usually show such wide ranges of variation that these sometimes cover the ranges of both parents, as seen in Fig. 7. In frost-hardiness, also, the same tendency can be recognized. In the present study, 3 hybrid families were intermediate, though two of these families were rather closer to either of the parental species. DIMPFLMEIER ('59) mentioned that hybrid progenies of larches showed their frost-hardiness close to that of the maternal trees though the paternal trees also played some rôle. But it seems difficult to draw any conclusions with regard to the inheritance of frost-hardiness of the larch species from only these limited data.

A question arises as to whether the early cessation of annual growth is connected closely with the poorness of the height growth of seedlings or not. The figures in KOBAYAKAWA's paper ('44) seem to suggest that the annual increment of height growth is influenced by the time of cessation of growth much more than by the quickness of height growth for L or G seedlings under conditions of different lengths of light periods. Sato *et al.* ('51) and Asada *et al.* ('65) also obtained results showing similar tendencies. In other words, the rise of frost-hardiness in a larch species or family is gained at the expense of height growth.

The average heights of the 3-year-old seedlings used in the present study are larger in H families than in L families (see Fig. 2). But the height growth of young larch plants is, in the stage of nursery stocks and even for those planted several years in forests, under a strong influence of various environmental factors (including nursery treatments) and probably under the influence of the stage of seed, also; that is to say, the true genetic differences of growth among the families or individuals possibly begin to reveal themselves in later stages of development or maturation. In fact, the average increments of height growth in the 3rd year of the above-mentioned seedlings are in order of (largest) Gk×L : L : L×Gs : L : L×Gk : Gk : Gk (smallest). These seedlings were obtained from nursery stocks to be supplied for ordinary use and were not treated to fit such a strict comparison as that under discussion here. On the other hand, it is also true that the hybrids between the dahurian and the Japanese larch show hybrid vigour in growth and are composed of individuals forming terminal buds much or a little earlier than the Japanese. A hope of breeding hardier larch varieties is left on this point. Inasmuch as all individuals of a hybrid family are not evenly hardy to frosts, attempts should be made to separate them into groups by the difference in the time of cessation of growth. Otherwise, it is necessary to make allowances for losses by frost damage to some extent for planting hybrid larches in some places, before we come to know exactly what crossings among the larch species and among their individuals will give the expected frost-hardiness to the progenies. With age, as mentioned above, the larch seedlings come to cease their annual growth earlier, and, in parallel with this, to express their frost-hardiness earlier, and also to raise their maximum hardiness to some extent. The seedlings or younger saplings should be therefore given full protection from frosts. This is more so because the younger plants are exposed to largest risks of frosts and/or drought in the first autumn or winter after planting in forests, especially in the case of planting in autumn (Takatoi *et al.* '64).

For future studies of frost-hardiness of the larches the following should be taken into consideration. The age and the growth condition of seedlings and the day-length condition, respectively, should be controlled to the same or be made common for all materials in comparing their hardiness. Especially it must be kept in mind that the day-length conditions do not always bring even changes on the cessation of growth in different latitudes. And for practical purposes it is repeated here that some of the phenological characters such as the time of terminal bud formation are the most reliable as criteria in the determination of first acquisition of frost-hardiness of the larch species or their hybrids.

Summary

The process of frost-hardening was studied on the top pieces of main annual shoots of 3-year-old seedlings of the dahurian (2 families) and the Japanese larch (2) and their reciprocal hybrids (3) in the autumn of 1965. Researches concerning their phenological characters and the increase and decrease of starch content in the tissues were also carried out.

From the freezing experiments, the following results were obtained:

1. Under conditions of day-length and temperatures at Yamabe, the dahurian larch seedlings (races from Saghalien and the South Kuriles) acquire hardiness to temperatures slightly lower than their freezing point or −5℃ at the end of September. The hardiness rises rather slowly from this point (with a short stagnation at −5℃) to −15℃ at the end of October when the minima of atmospheric temperatures fall approaching 0℃, and then it attains its maximum value to −25℃ at the end of November. On the other hand, the Japanese larch seedlings become hardy to −5℃ at the end of October about a month later than the dahurian. The hardiness rises rather rapidly and reaches nearly the same maximum value at nearly the same time as the dahurian. The hybrid families show indefinite tendencies in this respect, sometimes similar to either of the parental species, sometimes intermediate.

2. 4-hour freezing at −5℃ gives the seedlings which are still susceptible nearly the same extent of injury as that of freezing for 16 hours, at the middle and the end of September.

3. The process of (artificial) hardening at 0℃ for 2 weeks progresses in nearly the same rapidity as that under the natural condition of temperatures at Yamabe, early in autumn.

4. The excised pieces of annual shoots of 9-year-old saplings can usually withstand −30℃ at midwinter, and are obviously hardier than those of younger seedlings. But it is difficult to consider that the frost-hardiness of the larches, except the dahurian larch races from more northern localities, increases over this even in older ages.

Among the phenological characters, the time of terminal bud formation (b), the time of full suberization of annual shoots (s) and the time of colouring of needles (c) were to some extent characteristic to each family or species and were considered to be correlated to the process of rise of frost-hardiness. The excised pieces of shoots, either the treated or the control, had to be cultivated in water for a certain length of time, before the buds on them began to open. This length of time necessary for the bud-opening varied in a special relation with the stage of annual growth of the seedlings and with the dates of the experiments. On the materials cut off before a certain point of time (D) which is also characteristic to each family, however, the buds could not open.

Four changing points of starch content were recognized mainly in the bark tissue at the part 5 cm below the top: the respective beginning points of the increase (a^{cr}), the high constant (a^{max}), the decrease (a^{dec}) and the low constant stage (a^{min}).

Close correlations among the times of occurrence of these phenological or histochemical characters or between these and the process of frost-hardening are understood from Tab. 7 and Figs. 4 and 10. Especially the time of the terminal bud formation or the cessation of annual growth precedes the first acquisition of hardiness to −5℃ about 25~35 days in every family. And this relation is not changed even in the case of changing the time of terminal bud formation by artificial treatments such as the photoperiodic treatment.

The mechanism of frost-hardening in the larch species and hybrids also were discussed on the basis of these correlations and with reference to the literature.

No large difference between the Japanese (less hardy) and the dahurian larch (hardy) in their frost-hardiness is seen in the depth of winter. A difference is found only in the time of their first acquisition of hardiness to −5℃, and this time is significant only for discussing or comparing their hardiness against early frosts in some districts. At the same time, it is possible to increase the frost-hardiness against early frosts of larch seedlings to some extent by some means of utilizing their photoperiodic responses or the action of some kinds of chemicals. For the practical purpose of breeding hardier hybrids between these two species, however, no important genetic information could be obtained from the results of the present study. The only way left for the necessity of avoiding frost damage in the forests in Hokkaido where larches are to be planted at present is to separate individuals of hybrid progenies into groups by the difference of their time of terminal bud formation and to choose a district of climate sufficiently mild for each of these groups.

References

AOKI, K. (1955). On the artificial hardening of the twig of mulberry tree. Low Temp. Sci. Ser. B, **13**: 13–20.*

ASADA, S. (1958). Studies on the rest period of *Larix* MILL. Jour. Fac. Agr. Shinshu Univ., No. 8: 1–24.*

———, T. AKAI & N. TAKAHASHI (1965). 信州地方におけるカラマツ属の生長について（予報）. 76 回日林講. 234–236.**

DIETRICHSON, J. (1962). Breeding for frost resistance. Silv. Gen. **10**: 172–179.

DIMPFLMEIER, R. (1959). Die Bastardierung in der Gattung *Larix*. Forstwiss. Forsch. (Beih. Forstwiss. Centralbl.) Heft 12: 1–75.

EGUCHI, T., A. SAKAI, G. USUI & T. UEHARA (1966). Studies of selection of frost-hardy *Cryptomeria*, I. Silv. Gen. **15**: 84–89.

GLERUM, C. & J. L. FARRAR (1966). Frost ring formation in the stems of some coniferous species. Canad. Jour. Bot. **44**: 879–886.

GOTHE, H. (1952). Ein Kreuzungsversuch mit *Larix europaea* D.C., Herkunft Schlitz, und *Larix leptolepis* GORD. 1 Mitteil. Zeitschr. f. Forstgenetik. **1**: 108–110.

——— (1953). Ditto. 2 Mitteil. *Ibid.* **2**: 122–125.

HARADA, H. (1964). Translocation of minerals in larch seedlings in defoliation season. Jour. Jap. For. Soc. **46**: 124–126.*

HASEGAWA, K. & A. TSUBOI (1960). The effect of low temperature on the breaking of rest for winter bud in mulberry tree. Jour. Sericult. Sci. Jap. **29**: 63–68.*

ISHIDA, S. (1958). Freezing point of green Todomatsu-wood. Res. Bull. Coll. Exp. For. Hokkaido Univ. **19**: 123–144.*

JIMBO, T. (1936). Seasonal fluctuations of the osmotic value in plants. Biol. Rev. (生態学研究) **2**: 231–234.**

KOBAYAKAWA, S. (1944). The effect of length of day on growth of forest trees (I). Bull. Tokyo Imper. Univ. Forests **34**: 83–119.*

KOBAYASHI, A. (1952). 果樹の自発休眠の問題（解説）. 農及園 **27**: 1085–1090.**

KOMA, S. (1953). Studies on the rest period of the deciduous fruit trees (I). On the beginning, the ending and the depth of the rest period. Jour. Fac. Agr. Shinshu Univ., No. 3: 189–204.*

———, Y. MIYAZAKI & M. KITAZAWA (1955). Studies on the cold resistance of fruit trees. I. On the resistance of new shoots of deciduous fruit trees. Stud. Inst. Horticult. Kyoto Univ. (園芸研究集録). No. 7: 54–58.*

KURAHASHI, A., C. SASAKI & T. HAMAYA (1966). 開花期と積算温度. 北海道の林木育種 **9**-**2**: 20–27.**

LEVITT, J. (1951). Frost, drought and heat resistance. Ann. Rev. Pl. Physiol. **2**: 245–268.

MORITA, K. & A. SAKAI (1966). Studies on frost damage of poplars. I. Difference in frost hardiness among varieties of hybrid poplars. Jour. Jap. For. Soc. **48**: 267–273.*

OSTENFELD, C. H. & C. SYLACH LARSEN (1930). The species of the genus *Larix* and their geographical distribution. Kgl. Danske Vidensk. Selskab. Biol. Meddel. **IX**, 2: 1–107.

PARKER, J. (1963). Cold resistance in woody plants. Bot. Rev. **29**: 123–201.

SAKAI, A. (1955a). The relationship between the process of development and the frost hardiness of the mulberry tree. Low Temp. Sci. Ser. B, **13**: 21–31.*

——— (1955b). The seasonal changes of the hardiness and the physiological state of the cortical parenchyma cells of mulberry tree. *Ibid.* 33–41.*

——— (1956). The effect of temperature on the hardening of plants. *Ibid.* **14**: 7–15.*

——— (1957). Relation of increase of carbohydrate and water soluble protein to frost resistance in tree. *Ibid.* **15**: 17–29.*

——— (1958). The frost-hardening process of woody plant. II. Relation of increase of carbohydrate and water soluble protein to frost-resistance (2). *Ibid.* **16**: 23–34.*

——— (1959a). Ditto. III. Relation of low temperature to frost-hardening. *Ibid.* **17**: 29–34.*

——— (1959b). Ditto. IV. Relation of increase in sugar concentration to dehydration resistance. *Ibid.* 35–41.*

* In Japanese with English summary. **: In Japanese only.

SAKAI, A. (1959c). Ditto. V. The relationship between developmental stage and frost-hardening. *Ibid.* 43–49.*

——— (1960a). 植物における凍害機構の一端. 日本植物生理学会報 **1**: 21–26**.

——— (1960b). The frost-hardening process of woody plant. VII. Seasonal variations in sugars (2). Low Temp. Sci. Ser. B, **18**: 1–14.*

——— (1960c). Ditto. IX. The protective action of sugars against frost injury. *Ibid.* 23–34.*

——— (1962). Studies on the frost-hardiness of woody plants. 1. The causal relation between sugar content and frost-hardiness. Contrib. Inst. Low Temp. Sci. Ser. B, No. 11:1–40.

——— (1963a). 寒地と暖地の同一植物の間に耐凍性の差があるか. 北海道の林木育種 **5**-**2**: 5–7.**

——— (1963b). Survival of plant at super-low temperatures. III. The relation between the grade of frost-hardiness and the effective pre-freezing temperature. Low Temp. Sci. Ser. B, **21**: 1–16.*

——— (1964). The frost-hardening process of woody plant. X. The effective frost-hardening temperature in twig. *Ibid.* **22**: 29–50.*

——— (1965a). Survival of plant tissue at super-low temperatures. III. Relation between effective prefreezing temperatures and the degree of frost hardiness. Pl. Physiol. **40**: 882–887.

——— (1965b). 林木の耐凍性——寒さに強い系統を選抜する場合の 2, 3 の問題点. 北海道の林木育種 **8**-**2**: 5–8.**

——— (1966a). Temperature fluctuation in wintering trees. Physiol. Plant. **19**: 105–114.

——— (1966b). Studies of frost hardiness in woody plant. II. Effect of temperature on hardening. Pl. Physiol. **41**: 353–359.

——— & J. WADA (1963). Temperature variation in overwintering trees. Low Temp. Sci. Ser. B, **21**: 25–40.*

SATO, Y., M. MIYAKÔSHI & K. MUTO (1951). Factors affecting cold resistance of tree seedlings. (I). On the effect of period of photoperiodic treatment. Res. Bull. Coll. Exp. For. Hokkaido Univ. **15**: 63–80.*

SUGIMOTO, S. (1961). 長野県におけるカラマツ苗木の凍霜被害状況分析——被害苗木の活用と被害防止について. 北方林業 **13**: 91–96.**

TAKATOI, I. (1960). On the control of growth of Japanese larch seedlings. (I). Effects of rest time of height growth upon the frost hardiness of seedlings. Ann. Rep. Hokkaido Branch For. Exp. Stat. **1959**, 11–20.*

——— & T. WATANABE (1961). On the control of growth of Japanese larch seedlings. (II). On the progress of frost hardiness of seedlings, those height growth in controled by NAA, and photoperiodic managements. Ann. Rep. Hokkaido Branch For. Exp. Stat. **1960**, 19–30.*

——— & ——— (1962). 冬季におけるストローブマツとトドマツの耐凍性について. 北方林業 **14**: 16–18.**

———, ——— & U. KAMADA (1964). Frost damage on forest trees. (VI). The effects which transplanting in the autumn or needles-cutting in summer have on the frost hardiness of young Japanese larch trees. Ann. Rep. Hokkaido Branch For. Exp. Stat. **1963**, 108–118.*

TAMATE, M. (1927). からまつノ発芽及落葉期日ト海抜高トノ関係. 林業試験場気象彙報 9 号, 1–20.**

TERUMOTO, I. (1958). The relation between the cell sap concentration and the frost-hardiness of the table beet. Low Temp. Sci. Ser. B, **16**: 7–21.*

WATANABE, A. (1958). On the cold resistance of *Cinnamomum Camphora* SIEB. and *C. Camphora* var. *mominale* HAY. from Formosa. Bull Tokyo Univ. For. **54**: 63–69.*

YANAGISAWA, T. (1961a). Effects of seed source on seedling growth rhythm of Japanese larch. Ann. Rep. Hokkaido Branch For. Exp. Stat. **1960**, 65–70.*

——— (1961b). 主として気候上からみた本道への異郷土樹種の導入に関する考察. 北海道の林木育種 **4**-**2**: 12–24.**

カラマツ，グイマツ及びその種間雑種の耐凍性に関する研究（摘要）

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1965年秋季に，カラマツ2実生系，グイマツ（樺太系）2実生系及び両種の種間雑種3実生系の3年生苗の新条部を用いて耐凍性の増大過程を調べた。同じ材料について冬芽形成期（b），新条のコルク化完了期（s）及び黄葉期（c）の変異と，各組織内の澱粉量の消長（*a*）も調べた。

北海道大学低温科学研究所で凍結処理を行なった切枝による一連の実験によってこれらのカラマツ類の耐冬性について以下のことが明らかになった。

1. 東京大学北海道演習林山部苗畑での自然日長及び温度の条件の下では；グイマツ3年生苗は9月下旬にその凍結温度ないし −5℃ に対する耐凍性を獲得する。苗はその後ゆっくりとその耐凍性を増大し，10月下旬には −15℃ に耐え，次いで11月下旬以降には −25℃ に耐えるようになるが，これがこの苗（厳密には切枝）の耐凍性の最大値とみなされる。一方，カラマツ苗ははじめ全く耐凍性を示さず，グイマツより約1カ月遅れて10月下旬にやっと −5℃ に耐えるようになり，以後急速にその値をましてグイマツとほぼ同時にほぼ同じ最大値(僅かに劣るが)に達する。雑種カラマツ苗は実生系によって母系種または父系種に近い変化を示すか，あるいはその中間の変化をたどる。しかし毎回の実験における個体間の変異は両親種よりはるかに大きい。

2. 以上の実験では供試材料を16時間目的温度にさらしたが，9月中・下旬に行なった−5℃，4時間凍結実験の結果でも16時間処理と大差ないことがわかった。したがって野外でも比較的短い低温継続時間で霜害が生ずるものと考えられる。

3. 10月上・中旬に一部の材料を2週間 0℃ に保って hardening を行なったが，耐凍性の増大促進に効果が認められた。しかし，この効果は山部の同時期における野外温度低下に伴う自然増大とほぼ等しいはやさですすむものと思われる。

4. 9年生造林木の新条端は12月上・下旬において3年生苗のそれよりは明らかに耐凍性が大きく，−30℃ またはそれよりやや低い温度に耐える。カラマツ類（北限地域のグイマツ等を除外すれば）にとっては，恐らくこの程度が耐えうる最低温度であろう。

初めに述べた季節現象諸形質のうち，特に冬芽形成期の平均値は両種においてかなり異った値を示す。雑種実生系の値は両種の中間にあってその変異幅がはるかに大きい。また，切枝を温室内で水耕する際，凍結処理区・対照区共に冬芽の開舒に要する日数には一定の傾向が認められ，ある時期(D)以前に切取った材料では全く開舒しない。これは冬芽が休眠に入りその深さに変動

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があることと関係していると思われる。これに関連して開舒のためのいわゆる低温所要時数について若干の考察を行なった。

組織内の澱粉量の消長については特に新条下部の皮部に増大期（a^{cr}），最多期（a^{max}），減少期（a^{dec}），及び最少期（a^{min}）を認めることができる。

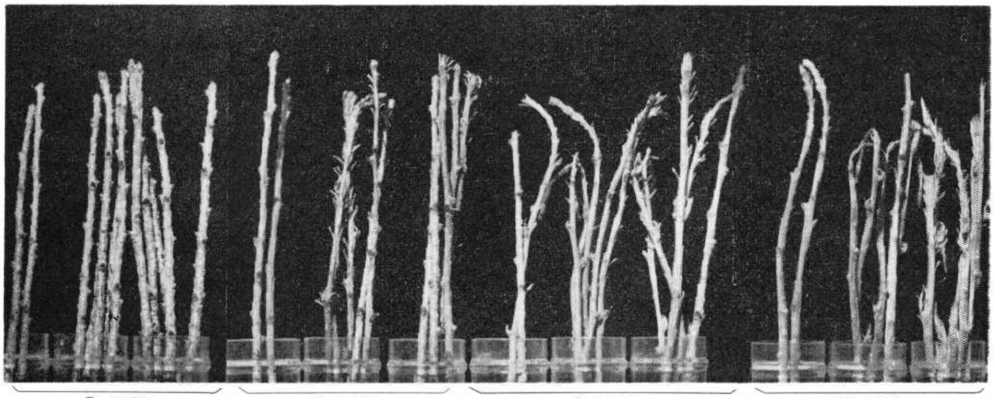
これらの諸形質の発現・変化の主要時点と耐凍性増大過程には著しい一致が認められる（第7表，第4・10図）。中でも冬芽形成期あるいは当年伸長生長停止期はいずれの実生系においても−5℃ 耐凍性発現の 25〜35 日前にある。しかもこの関係は日長処理，ある種の薬品処理あるいは苗令の増加によって生長停止がある程度まで早まる（あるいはおくれる）場合においても変らない。したがってこの時期に植物体内に重要な生理的変化が行なわれるものと推定されるが，これとの関連において，この研究の結果と一部文献に基づいてカラマツ類及びその雑種類の耐凍性増大過程に関する推論・考察を行なった。

従来の知見及びこの研究によって，カラマツとグイマツの耐凍性の違いは厳寒期よりもむしろ初霜時における耐性の有無によって示されることが明らかになった。しかし雑種カラマツ類の耐凍性の遺伝的傾向についてはほとんど知られていない。今後，耐凍性雑種カラマツ類を育成しようとする場合，まずその生長停止ないし冬芽形成における光週反応及びその遺伝性について調査研究することが必要である。当面する雑種カラマツ苗の実際造林に際しては，冬芽形成の早遅によって同一実生系内の苗を類別し，それぞれの光週性を考慮して植栽地を異にするのが最も安全な方法であろう。

(1967 年 2 月稿)

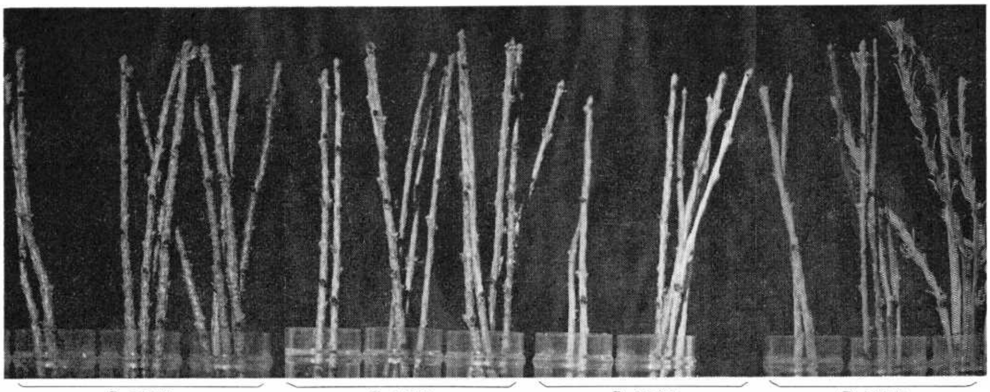
PLATE

Frost injuries on the 2-year-old Japanese larch seedlings (see Table 3 in the text).
Photo: Nov. 20, 1965.



S-1197 S-1206 S-1207 S-1210

Materials frozen at $-5°C$ on the 2nd date (Sept. 15).
Photo: 5 days after cultivation in water. From left: control, frozen for 4 hrs. and for 16 hrs., respectively.



S-1197 S-1206 S-1207 S-1210

Materials frozen at $-5°C$ on the 3rd date (Sept. 25).
Photo: 5 days after cultivation in water. From left: control, frozen for 4 hrs. (except S-1207) and for 16 hrs., respectively.