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Studies on Frost Hardiness of *Pinus contorta* Dougl. Seedlings Grown in Climate Chambers

Studier över frosthärdighet hos fröplantor av Pinus contorta Dougl. odlade i klimatkammare

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Abstract

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Pinus contorta populations from latitudes 47° to 63°N were cultivated in climate chambers and freeze tested at varying photoperiods. The results were compared with data from field trials comprising other populations from the same latitudinal range. A good agreement between our data on frost hardiness from the climate chambers and the ones from field trials was obtained. Breeding for frost hardiness in *Pinus contorta* ought to be done by early testing using the design developed in the present investigation. The longer the night length the greater the hardiness. Low temperature during the night promotes the development of hardiness. The more northern the origin the shorter the night length required to induce hardiness. Dry matter content of the upper 3 cm of the shoots and the lengthening of the secondary needles are strongly correlated with hardiness.

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1 Introduction

Since the discovery by Sylvén (1940) that the photoperiod plays a major role for growth in *Picea abies*, *Pinus sylvestris* and *Populus tremula* many investigations have been designed to study the photoperiodic influence on growth and growth cessation. The photoperiodic responses are determined by the length of the night. To bring about a long night response the night must not be interrupted by light (intervals) (Wareing 1950, Olson & Nienstaedt 1957, Nitsch 1962, Dormling et al. 1968). The photoperiodic response for growth cessation varies within a tree species. Plants originating from northerly or high altitude regions are adapted to stop growth at shorter night lengths than are plants from lowland or southerly regions (Sylvén 1940, Langlet 1943, Johnsson 1951, Pauley & Perry 1954, Robak 1957 and 1962, Vaartaja 1959, Holzer 1966 and 1979, Dormling et al. 1968, Morgenstern 1969, Magnesen 1969 and 1972, Dormling 1971, 1973, 1977 and 1979, Heide 1974, Holzer & Nather 1974, Håbjørg 1977, Kuser & Ching 1980). Height growth cessation and bud set in response to declining photoperiods are evidently an indirect adaptation to the environment, which allows plants and trees to reach the resting stage before the occurrence of the first autumn frost.

Even if the photoperiod is the basic factor that triggers the processes leading to bud set and lignification, temperature exerts an important influence on these processes too (e.g. Dormling 1977 and 1979, Heide 1977, Rosvall-Åhnebrink 1977, Sandvik 1978).

Introduction of a foreign tree species ought to be preceded by a basic investigation of the photo- and thermoperiodic responses of its populations. This will not eliminate the need for field trials but these

could probably be more efficiently designed than without the basic knowledge. Thus, based on the results obtained in climate chambers, the field trials might be concentrated to the promising provenances. Moreover, the location of the field trials could be better adjusted to the climatic variation in the country.

This is supported by the results presented by Larsen (1978a—d). He showed in a convincing way the usefulness of freeze testing seedlings of *Abies grandis* and *Pseudotsuga menziesii* following cultivation under controlled conditions. The feasibility of using controlled environment cabinets in forest genetic research is also evident e.g. from the studies of Pollard & Ying (1979).

Besides that, experiments under controlled conditions may give invaluable information on the possibilities of using certain plant traits in early tests. This in turn probably has its greatest value for studies of the inheritance of frost hardiness and growth capacity which will be emphasized in designing the future breeding of the species under study.

During the sixties and seventies the Swedish forest companies became increasingly interested in *Pinus contorta*. Some series of field trials with provenances were established during this time (Hagner & Fahlroth 1974, Lindgren et al. 1976, Persson et al. 1979). The detailed study of the growth rhythm performed by Hagner & Fahlroth (1974) showed that the growing season of *Pinus contorta* was longer than that of *Pinus sylvestris*; in addition, the daily growth was greater in *P. contorta* than in *P. sylvestris*. Moreover, the most northerly provenances of *P. contorta* seemed to be harder than autochthonous provenances of *P. sylvestris*.

Since some provenance series of field

trials of *P. contorta* are already established, it may be argued that basic photo- and thermoperiodic investigations carried out under controlled conditions would not be of any practical value for the breeding of this species. However, a basic investigation may disclose whether or not there is a clinal variation with respect to hardiness and growth rhythm. The critical temperature above which no frost damage is induced as well as the relationship between temperature and degree of frost damage may also be determined. It has to be remembered that field trials always have the drawback that the critical years with extreme weather conditions appear randomly. This means that some of the trials will escape exposure to critical conditions. This in turn may lead to a misinterpretation of the suitability of the genetic entries on certain field trials.

Besides, traits of great significance at a young age may be revealed, which may be used in breeding and practice.

Some results from our studies on the behaviour of *P. sylvestris* and *P. contorta* in response to photo- and thermoperiod were briefly reported earlier (Dormling 1971 and 1975, Dormling et al. 1977a and b, Ekberg et al. 1979, Jonsson et al. 1979, Jonsson 1980).

The purposes of the present investigation may be summarized as follows:

1. To determine the photo- and thermoperiodic requirements of different populations for induction of frost hardiness.
2. To examine several characters to see if any could be feasible for tests at a young age.

2 Material and Methods

2.1 Material

In Table 1 the geographic data of the populations included in different experiments of the present investigation are given (see also Figure 1).

For some of the populations the seed amount was not large enough to permit their testing in more than one experiment. In these cases they were replaced by populations of similar origin.

2.2 Methods

2.2.1 Cultivation

Gravel mixed with sand and perlite (2:1:1) was used as substrate in experiment I. In experiments II—V this substrate was replaced by mineral wool, which seemed to

give a better control of the water and nutrient supply.

In all experiments a complete nutrient solution (Ingestad 1979) of low concentration (100 mg N/l) was given once or twice a day, the proportions of N:K:P amounting to 100:65:13. The air humidity was kept at 75 per cent relative humidity. If otherwise not stated, the light intensity at seedling level was 22,000 lux (70W . m⁻², 270—695 nm).

2.2.2 Treatments

Five experiments were conducted. The purpose of them may be summarized as follows:

- I For the first time to study the photo- and thermoperiodic response of several morphological traits.

Table 1. Data on the origin of the populations studied in experiments I—V.

Species	No.	Name	Lat. °N	Long.	Altitude m	I	II	III	IV	V	
<i>P. contorta</i>	16	Tagish	YT	62°08'	135°18'	620				●	●
	15	Fireside	BC	59°40'	127°08'	565				●	●
	2	Yakutat	AK	59°30'	139°10'	45	●	●	●		
	14	Trutch	BC	57°40'	122°55'	825				●	●
	13		BC	56°47'	121°48'	1000				●	●
	12	Chetwynd North	BC	55°45'	121°40'	670				●	●
	4	Nass River	BC	55°37'	128°38'	300		●	●	●	●
	11	Fort St. James	BC	55°08'	124°10'	980				●	●
	17	Pewsnip	BC	55°08'	128°00'	730					●
	10	Telkwa	BC	54°39'	127°03'	780				●	
	9	Clucutz Lake	BC	53°50'	123°30'	730				●	●
	8	Valemount	BC	52°50'	119°13'	850				●	●
	7	Miller Lake	BC	50°37'	119°48'	991				●	●
	1	St. Regis	MT	47°22'	115°24'	945	●	●	●	●	●
	3	Long Beach	WA	46°26'	124°03'	15		●	●		
<i>P. sylvestris</i>	6b*	Vuostohangas, Pajala		67°15'	23°22'	260			●		
	6a*	Korpilombolo		66°55'	23°03'	190	●	●			
	5	Ventzelholm		57°45'	15°37'	190	●	●	●		

* Number 6 stands for two different populations (a, b), alternating in experiments I, II and III.

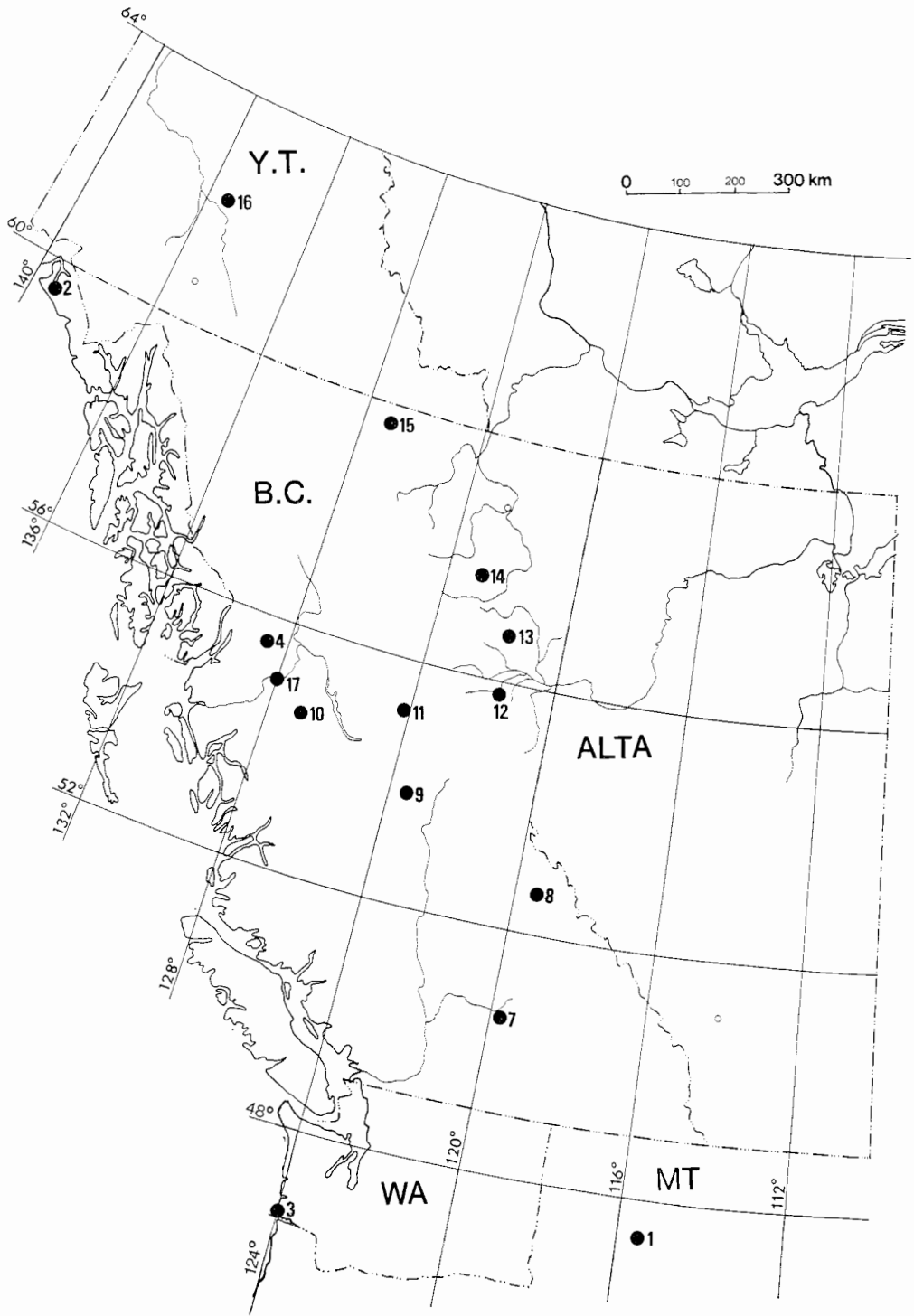


Figure 1. The origin of the populations studied in the present investigation.

- II To study the frost resistance of four populations of *Pinus contorta* cultivated under different photo- and thermoperiodic conditions. To develop a technique for freeze testing.
- III Freeze testing to -10°C of the same populations as in experiment II. In contrast to experiment II the plants were cultivated under a gradual lengthening of the night.
- IV To study the genetic variation in frost hardiness at certain photoperiods following cultivation with a gradual lengthening of the night. Freeze testing performed at night lengths 13 and 14 h during the first growth period and at a night length of 13 h during the second growth period.
To study the relationship between frost damage on the one hand and dry matter content in the apical shoot (sometimes also in lateral shoots), lengthening of secondary needles and anthocyanin colour on the other hand.
- V This experiment had to be carried out as a complement to experiment IV to obtain results from freeze testing at shorter night lengths, in this case 11 and 12 h.

Experiment I

During the two first weeks after sowing the night lengths were 6 and 8 h for the northern and the southern material, respec-

tively. The thermoperiod was $25/15^{\circ}\text{C}$. After that the material was cultivated under the following light and temperature conditions for 26 weeks:

Photoperiods:

20/4, 18/6 and 16/8 h of light/darkness.

Thermoperiods:

P. contorta — combinations of day/night temperature $25/25^{\circ}\text{C}$, $25/15^{\circ}\text{C}$, $25/10^{\circ}\text{C}$, $25/5^{\circ}\text{C}$, $20/20^{\circ}\text{C}$ and $20/10^{\circ}\text{C}$.

P. sylvestris — $25/15^{\circ}\text{C}$, $25/5^{\circ}\text{C}$ and $20/10^{\circ}\text{C}$.

Each population was represented by 16 plants per treatment.

Experiments II—V

The design of experiments II—V is indicated in Table 2. Some additional information is given below.

Period No. 2: To get plants large enough for the measurement of different characters, material in all experiments was cultivated until all the seedlings had developed top buds and secondary needles, which took eight weeks.

Period No. 3: The full light intensity as given above was applied for 8 h per day (cf. Figure 2). The light intensity used during the light interval of the remaining 16 h, as well as the arrangement of the night (darkness), are apparent from the same figure.

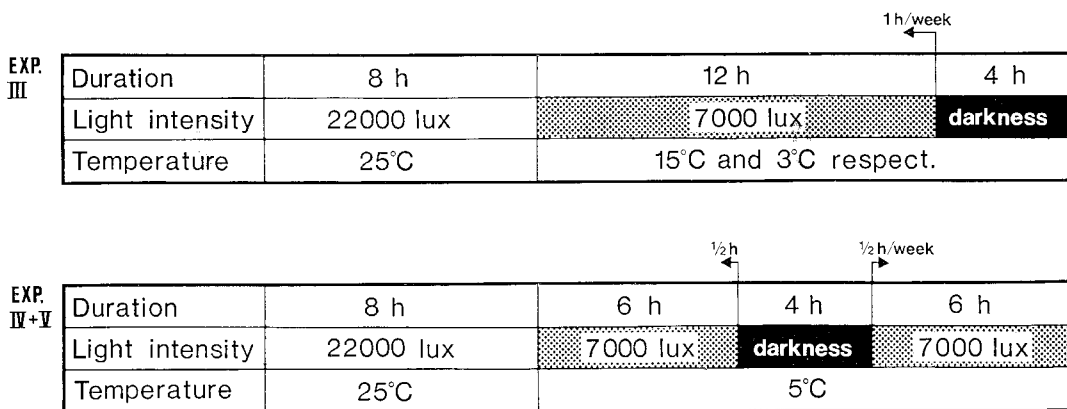


Figure 2. The duration of the periods with various light and temperature conditions during a day in period No. 3 (cf. Table 2) in experiments III—V. The arrows illustrate how the night was prolonged. In experiment III the temperature of $+3^{\circ}\text{C}$ was given to one half of the plants during the last four weeks preceding the freezing tests.

Table 2. The conditions of treatments (night length in hours and day/night temperatures in °C) during the different periods of the experiments as well as the duration of the periods.

Experi- ment	Period					
	1	2	3	4	5	6
	Cultivation of sown material	Cultivation of potted material	Treatment for growth cessation and hardening (autumn)	Freezing test	Prolonged autumn	Cultivation under conditions stimulating growth
	2 weeks	8 weeks	3—12 weeks	1 night	2 weeks	4—6 weeks
II	8 h 20°C	4 h 25/15°C	6, 8, 10, 12, 14 and 16 h 25/20°C and 25/10°C Freezing tests were carried out after 3 and 9 weeks at each photoperiod.	16 h - 10°C for 3—4 h Gradual freezing to - 10°C and successive thawing to + 10°C	Light and temperature conditions as before freezing	— — — —
III	8 h 20°C	4 h 25/15°C	Gradual prolongation of the night by 1 h a week for 12 weeks 25/15°C; 25/3°C the last 4 weeks before freezing. Samples for frost exposure were taken at the end of the periods with 8, 10, 12, 14 and 16 h of darkness respectively.	16 h - 10°C for 3—4 h Gradual freezing to - 10°C and successive thawing to + 10°C	Light and temperature conditions as before freezing	Continuous light 20°C
IV	6 h 20°C	4 h 25/15°C	Gradual prolongation of the night by 1 h a week for 10 weeks from 4 h to 14 h 25/5°C Samples for frost exposure were taken at the end of the periods with 13 and 14 h of darkness respectively.	16 h - 10°C for 3—4 h Gradual freezing to - 10°C and successive thawing to + 10°C	Light and temperature conditions as before freezing	Continuous light 20°C
V	6 h 20°C	4 h 25/15°C	Gradual prolongation of the night by 1 h a week for 8 weeks 25/5°C Samples for frost exposure were taken at the end of the periods with 11 and 12 h of darkness respectively.	16 h - 10°C for 3—4 h Gradual freezing to - 10°C and successive thawing to + 10°C	Light and temperature conditions as before freezing	Continuous light 20°C

In contrast to the previous experiments, a gradual lengthening of the night was applied in experiments III—V. From the original length of 4 h, the night was weekly

prolonged by 1 h (cf. Figure 2 and Table 2). The gradual lengthening of the night implies that the duration of the treatment with a particular night length was only one

week, in contrast to the design of experiment II, in which the same treatment lasted for 3 and 9 weeks, respectively.

Period No. 4: The freezing was carried out on trucks (Figure 3), adapted so that only the upper parts of the plants were exposed to the frost, while the root systems were protected. Before and after freezing the plants were placed at +10°C for 3—5 h in order to bring about a more gradual transfer between drastically differing temperatures.

Period No. 5: After two weeks in conditions identical with those before freezing, a classification of frost injuries was performed.

Period No. 6: As the pine species investigated are able to start growing without a treatment to break dormancy (cf. Dormling et al. 1977b), the material was directly transferred to favourable conditions for an investigation of the consequences of frost injuries on survival and growth of the plants.

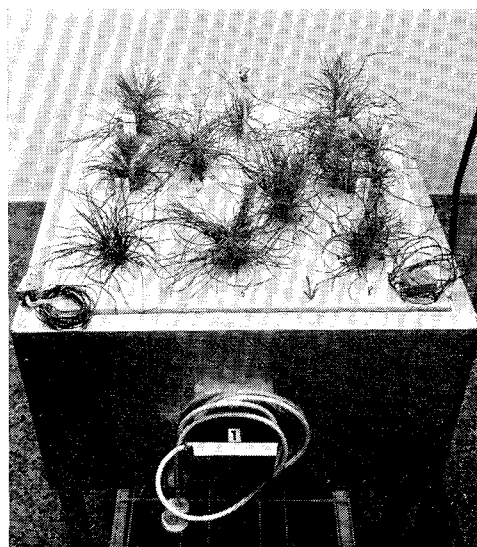


Figure 3. A truck adapted for freezing the upper parts of the plants while the root systems are protected from freezing.

Experiment IV, second growth period

A part of the material in experiment IV was intended for testing during its second growth period. After period No. 3 this material was exposed to an artificial winter consisting of a series of treatments with long nights and low temperatures (cf. Table 3).

Table 3. The treatments constituting an artificial winter.

Day/night temperature °C	Night length h	Weeks of treatment
25/5	16	2
15/5	16	5
2 (constantly)	24	1
15/5	16	1
25/15	4	2

After that, the material was treated in parallel to that of experiment V, starting with period No. 3 (see Table 2). It was tested with respect to frost hardiness after exposure to 13 h night.

2.2.3 Characters studied

In experiment I, several characters were studied to get information about their dependence on photoperiod and thermo-period. Height, bud setting and development of secondary needles were studied continuously during 26 weeks. At the end of this experiment 16 additional characters were recorded (cf. Table 6).

In experiments II—V bud setting was observed three times a week and plant height was measured once a week during period No. 2. The height growth of plants as well as the growth of their secondary needles was studied during period No. 3.

Lengthening of secondary needles

The growth of secondary needles (2 labelled needles/plant) was measured once a week during period No. 3 until the freezing test took place. On the freeze-tested plants, at

least three measurements were performed at the end of this period, which allowed calculation of the growth of the secondary needles during the two last weeks preceding each freezing test.

Frost hardiness

As a measure of hardiness attained under various photo- and thermoperiodic treatments, the extent of frost injuries after a gradual freezing to -10°C was used in experiments II—V.

The severity of frost injuries was classified in classes 0—5 as follows:

- 0 = no visible injury
- 1 = secondary needles slightly injured
- 2 = secondary needles extensively injured (yellow-brownish colour), primary needles almost undamaged
- 3 = secondary needles severely injured, primary ones injured over one half or more of their length
- 4 = both primary and secondary needles severely injured; injured, but living tissue at the top of the stem
- 5 = dead top, the whole plant severely injured, almost no green needles

The extent of injuries was expressed in different ways. *The percentages of plants in classes 4 and 5* were pooled to get information about the ability to survive of the material under study.

In the field, even injuries classified as 3 will probably influence the survival of plants. For this reason, *percentages of plants in classes 3—5* were calculated as well. *Mean frost damage* calculated for each particular population gave information about occurrence of all types of injury, even the slight ones. A disadvantage of this type of expression was that it was uncertain whether or not the scale was linear.

Dry matter content

Simultaneously with freezing tests, estimations of the dry matter content were performed on material treated in parallel with the tested plants. Approximately 3 cm of the *apical* shoots were harvested, weighed and dried at 70°C for 40 h. Dry matter content in per cent was calculated.

The correlation between the dry matter content of the apical shoot and the lateral shoots was highly significant (cf. Table 7). It was therefore possible to use *lateral* shoots for dry matter estimations. An advantage associated with this procedure was that the same plants could be used for dry matter estimations as well as for freezing tests. A disadvantage was that not all plants produced laterals large enough for use.

Growth after freezing

The influence of frost injuries upon the survival and subsequent growth of plants was investigated by exposing the plants to favourable conditions (20°C and continuous light). Measurements and classification of plants were performed 4, 5, and 6 weeks after freezing.

Photographic recording of the appearance of the frozen plants has been shown to be a very valuable way of registering injuries and growth. This was especially important since consistency of classification had to be maintained for several months when comparing plants investigated in different experiments with the same pattern of treatment.

Intensity of anthocyanin colour

During the period of artificial winter (cf. Table 3) the intensity of anthocyanin colour in secondary needles was classified on a scale from 0 to 3 (0 = no visible violet colour, 3 = very intense colour).

3 Results and Discussion

3.1 Hardiness and environmental conditions

3.1.1 The role of the photoperiod in the development of hardiness

As pointed out in the introduction, cessation of growth and the development of hardiness in the temperate zone tree species are supposed to be controlled mainly by the photoperiod. The reason for this is that

photoperiod is the only factor varying in the same way each year. Therefore, in experiments II and III the main aim was to study the influence of photoperiod on the development of frost hardiness in some populations of *P. contorta* and *P. sylvestris*. Simultaneously the influence of the thermoperiod was tested to some extent.

In Figure 4 mean values of frost damage

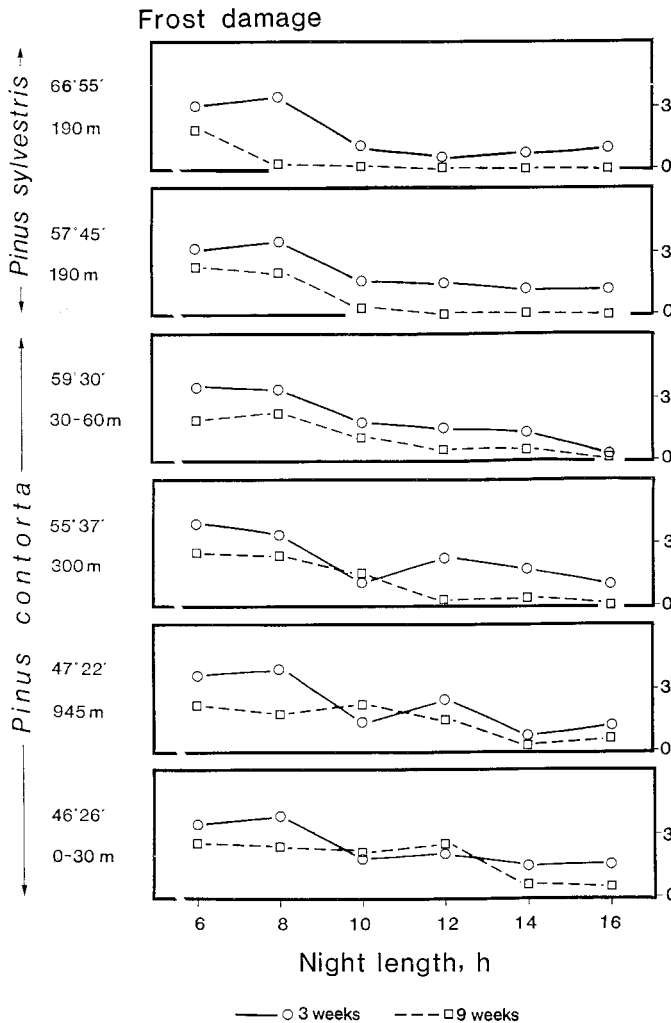


Figure 4. Experiment II. Frost damage (n = 8) after freezing to -10°C following parallel treatments with six different night lengths for 3 and 9 weeks respectively. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud (cf. chapter 2.2.3).

after freezing to -10°C are shown separately for the six populations at the six different photoperiods used in experiment II. A clearcut influence of the photoperiod is seen in this diagram. The longer the night, the lesser the extent of injuries observed after freezing. The effect of long night increased with the duration of the treatment. Thus, 9 weeks of treatment resulted in a higher degree of hardiness than 3 weeks of treatment. This agrees with the results reported by Aronsson (1975) and Christersson (1978).

Mean frost damage induced by -10°C at five different night lengths in experiment III are illustrated in Figure 5. In this experiment a gradual prolongation of the

night length was applied at two night temperature regimes (cf. Table 2). The results agree with the ones from the previous experiment.

In Figure 6 another parameter for estimating the frost damage noted in experiment III is used, viz. percentage of plants in classes 4–5. This parameter may give a better indication of the practical consequences of early frosts in the autumn than mean values for frost damage. Injuries of this severity (classes 4–5) are supposed to be closely connected with plant death. The trend towards decreasing percentages of severely injured plants with increasing night length is evident.

The results obtained in experiments II

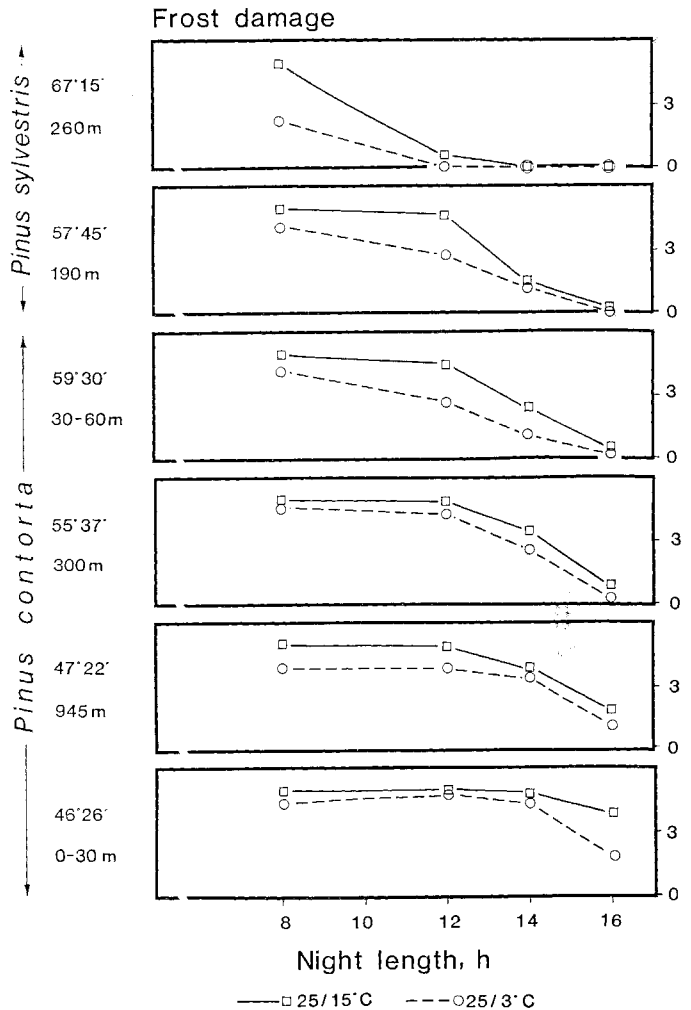


Figure 5. Experiment III. Frost damage ($n = 10$) in plants frozen to -10°C after treatment with day/night temperatures $25/15^{\circ}\text{C}$ and $25/3^{\circ}\text{C}$ respectively and a gradual prolongation of the nights (1 h/week). Freezing tests were performed after one week with the night lengths indicated. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud.

and III were important for the planning of our further studies. They showed that the photoperiod plays a crucial role in the hardening process. This agrees with the results reported by Hagner (1970b) following freeze testing at -30°C of two provenances of *P. contorta* originating from latitudes 49° and 57° . In the following experiments the main aim was to investigate genetic differences in photoperiodic reactions between populations of *P. contorta* of varying origin.

The data obtained in experiments II and III suggested that the best possibility of distinguishing different photoperiodic reac-

tions of the populations would be to test them at a night length close to 12 h. Night lengths varying between 11 h—14 h were therefore used in experiments IV and V.

A conspicuous difference in frost hardness between materials frozen after treatment with varying night lengths, 11—14 h, was noted. The material treated with 11 h night was severely damaged, only occasional plants having survived, while the treatment with 14 h night resulted in frost tolerance as indicated by good survival and negligible injuries.

The diagrams in Figures 7—9 show frost damage in various populations treated with

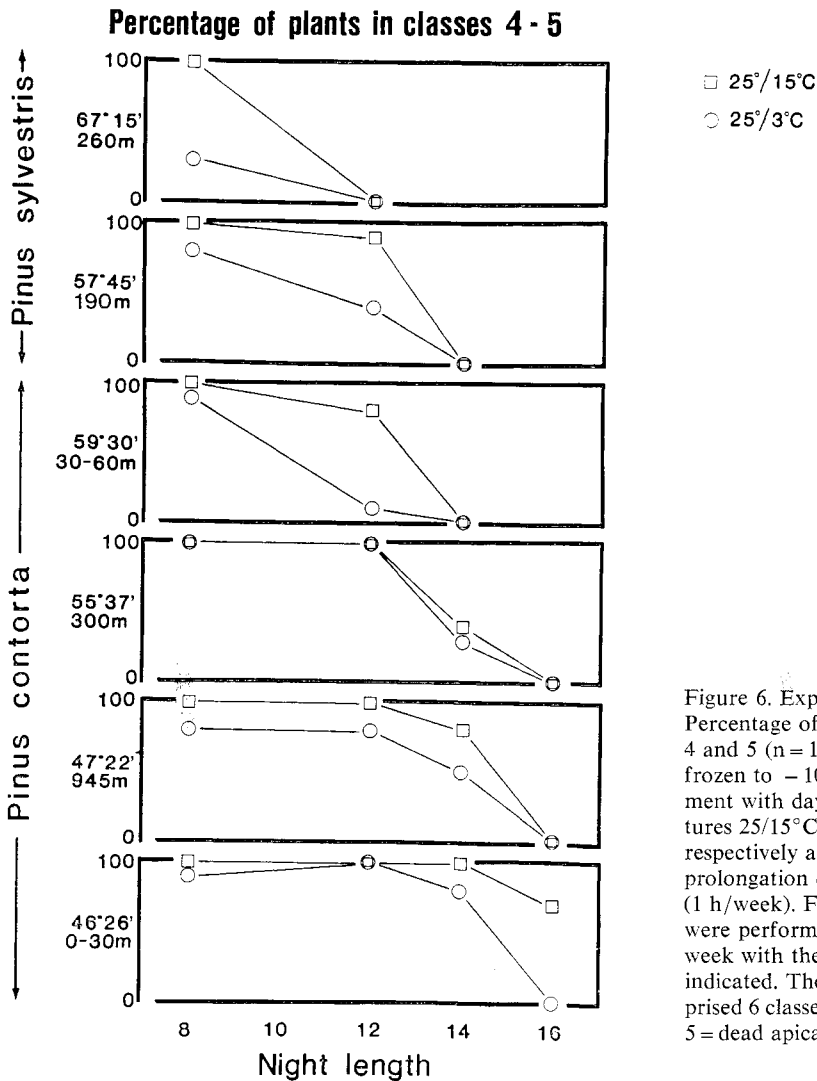


Figure 6. Experiment III. Percentage of plants in classes 4 and 5 ($n = 10$) in material frozen to -10°C after treatment with day/night temperatures $25/15^{\circ}\text{C}$ and $25/3^{\circ}\text{C}$ respectively and a gradual prolongation of the nights (1 h/week). Freezing tests were performed after one week with the night lengths indicated. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud.

four night lengths. The influence exerted by the photoperiod on the development of hardness is apparent when comparing the values for the same population at different night lengths.

The material frozen after treatment with 11 h night showed great frost injury, whereas only slight injury was noted at a night length of 14 h.

These night lengths may seem relatively long. However, it should be noticed that the frost injuries demonstrated here reflect the degree of hardness built up during all parts of the photoperiodic treatments including those with the given night length rather than the plant reaction at this particular night length. The fact that the effect of a particular night length is probably somewhat delayed leads to the presumption that the night inducing the start of hardening was shorter. Owing to the gradual prolongation of the night in these experiments, it was impossible to establish the exact length of the night which induces hardness. A direct translation of the night lengths under growth chamber conditions to outdoor conditions is not possible, since the outdoor photoperiodic conditions cannot be exactly simulated.

The genetic variation, which is revealed in Figures 7—9, will be discussed in chapter 3.2.

3.1.2 *The role of the thermoperiod in the development of hardness*

To some degree, the influence of thermoperiod was studied in experiments II and III. It has been shown that the difference between the extent of frost injury after treatment with 20°C and 10°C was not significant, which permitted a pooling of the materials in the calculations.

One possible explanation of the above-mentioned non-significant difference might be that the critical value which provokes a positive influence on hardening is below +10°C. This was why the night temperatures were lowered in experiment III to 15°C and 3°C, respectively. As may be seen from Figures 5—6, a night temperature of

+3°C provoked hardening more readily than a night temperature of +15°C. The critical value of the temperature below which the hardening process is favoured must therefore be found in the interval between +10°C and +3°C.

Based on the results in experiments II and III, a night temperature of +5°C was applied in experiments IV and V. This temperature ought to be low enough to stimulate the development of hardness.

Summary. Summarizing the results discussed in this chapter it may be stated that the longer the nights, the higher the degree of hardness. This was true both in experiment II, in which the materials were treated in parallel with various photoperiods for a relatively long time, and in experiments III—V, in which a gradual prolongation of the night was used.

The photoperiod is apparently the factor inducing the start of the hardening process. However, there are other factors influencing the development of hardness. It was shown that low night temperatures stimulate this process. A night temperature below +10°C is favourable for hardening.

3.2 Genetic variation in hardness

3.2.1 *Clinal variation in frost hardness*

The data obtained in experiments II—III, illustrated in Figures 4—6, indicate that there are large genetic differences with respect to hardness between the populations tested.

Since only four populations were tested in experiments II and III, there was no possibility of proving any clinal variation in them. In each of experiments IV—V, 12 populations, with a latitudinal range of approximately 15°, were tested. Freeze testing at four photoperiods, 11—14 h night lengths, would give good opportunities to reveal a clinal variation, if it exists at all.

The results from the freezing tests are illustrated in two ways in Figures 7 and 8. Independently of the parameters used, a clinal variation is seen for the treatments 12 h and 13 h. No clinal variation can be

revealed for treatments giving rise to low percentages of damage (14 h) or close to 100 % injury (11 h).

The clinal variation becomes still more apparent when all injuries, even the slight ones, are considered as may be seen from Figure 9, in which mean frost damage is illustrated.

As may be seen from Figures 7—9, latitude exerts a strong influence on the degree of frost hardiness reached at a certain photoperiod. Besides that the altitude also seemed to play a role.

The results from the freezing test during the second growth period are illustrated in Figure 10. The strong latitudinal influence on percentage of plants in classes 4—5 is evident also in this case.

3.2.2 Regressions of frost hardiness on geographic variables

These results called for a stepwise regression analysis to test the relative importance of latitude and altitude in the development of frost hardiness. Five independent variables, latitude, altitude, latitude², altitude² and latitude×altitude, were included in the regression analysis. Percentage of plants in classes 4—5 and 3—5 as well as mean frost damage were used as dependent variables (cf. Material and Methods).

The results obtained are presented in Table 4. As seen in this table, the R²-values are high, which means that the genetic differentiation between the *P. contorta* populations is substantially explained by

Percentage of plants in classes 4-5

Pop No.	Lat.	Alt. m	11 h			12 h			13 h			14 h		
			0	50	100	0	50	100	0	50	100	0	50	100
16	62° 08'	620	0	50	100	0	50	100	0	50	100	0	50	100
15	59° 40'	565	0	50	100	0	50	100	0	50	100	0	50	100
14	57° 40'	825	0	50	100	0	50	100	0	50	100	0	50	100
13	56° 47'	1000	0	50	100	0	50	100	0	50	100	0	50	100
11	55° 08'	980	0	50	100	0	50	100	0	50	100	0	50	100
17	55° 08'	730	0	50	100	0	50	100	0	50	100	0	50	100
12	55° 45'	670	0	50	100	0	50	100	0	50	100	0	50	100
4	55° 37'	300	0	50	100	0	50	100	0	50	100	0	50	100
10	54° 39'	780	0	50	100	0	50	100	0	50	100	0	50	100
9	53° 50'	730	0	50	100	0	50	100	0	50	100	0	50	100
8	52° 50'	850	0	50	100	0	50	100	0	50	100	0	50	100
7	50° 37'	1000	0	50	100	0	50	100	0	50	100	0	50	100
1	47° 22'	945	0	50	100	0	50	100	0	50	100	0	50	100

Figure 7. Experiments IV and V. Percentage of plants in classes 4—5 (n = 15) after freezing to -10°C at night lengths 11, 12, 13 and 14 hours respectively. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. The populations are arranged according to their decreasing latitude, with the exception of the four populations from the 55th parallel, in which case the altitude was decisive for ranking. Populations Nos. 17 and 10 alternate in experiments IV and V.

Percentage of plants in classes 3-5

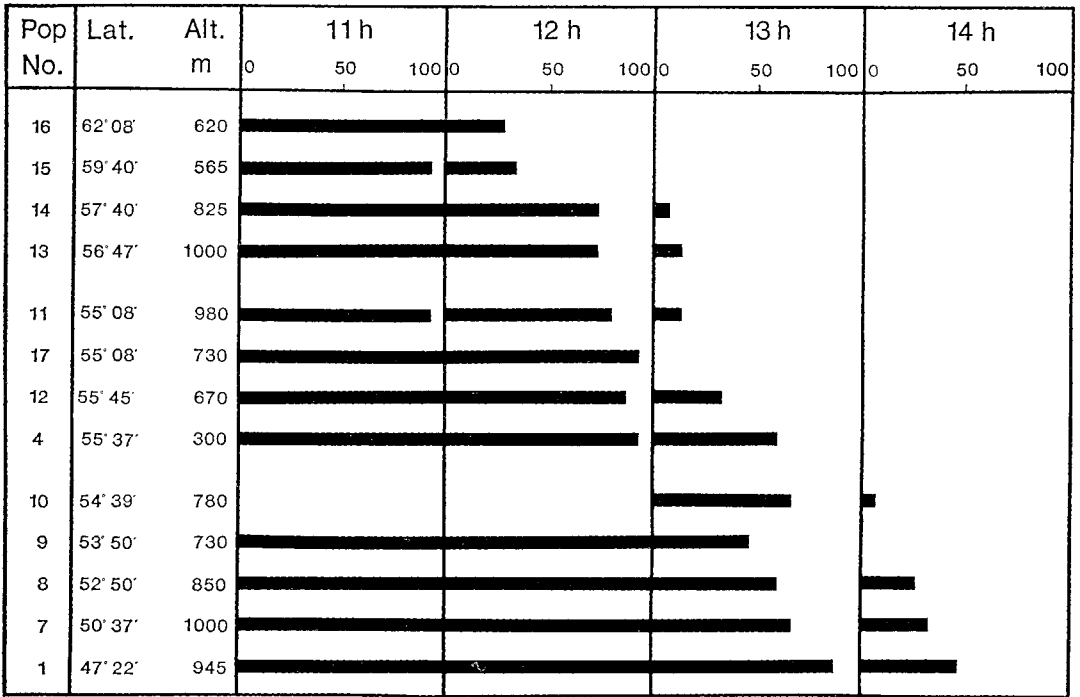


Figure 8. Experiments IV and V. The same as Figure 7 but frost damage estimated as plants in classes 3—5.

Frost damage score following freezing at -10°C.

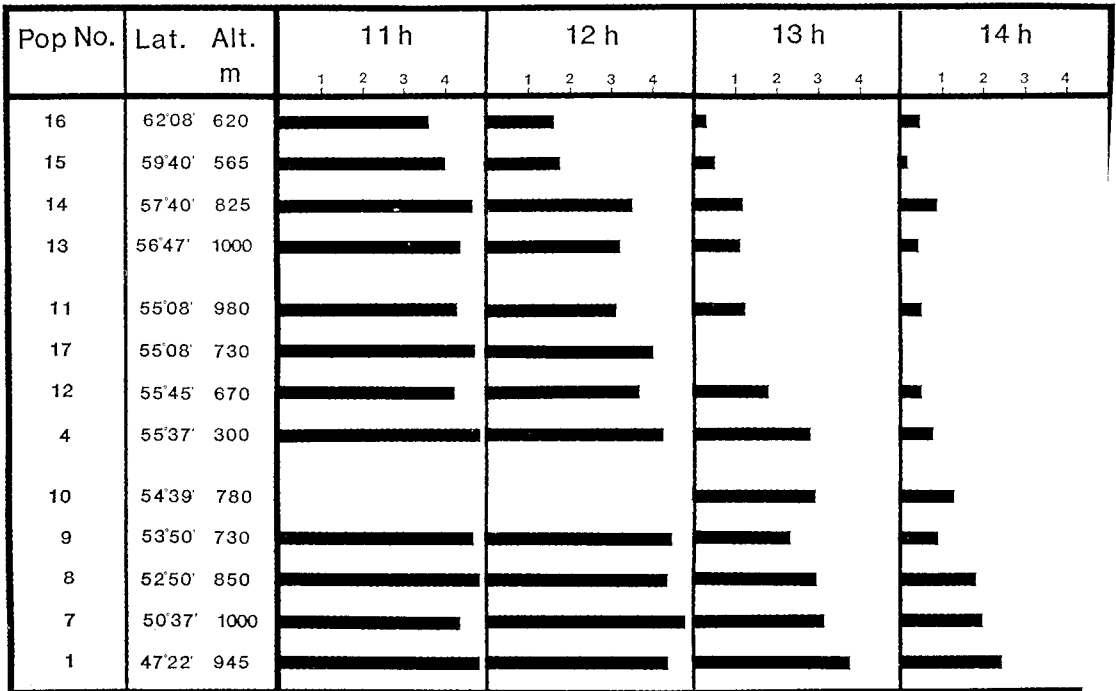


Figure 9. Experiments IV and V. The same as Figure 7 but the effect of freezing estimated as mean frost damage.

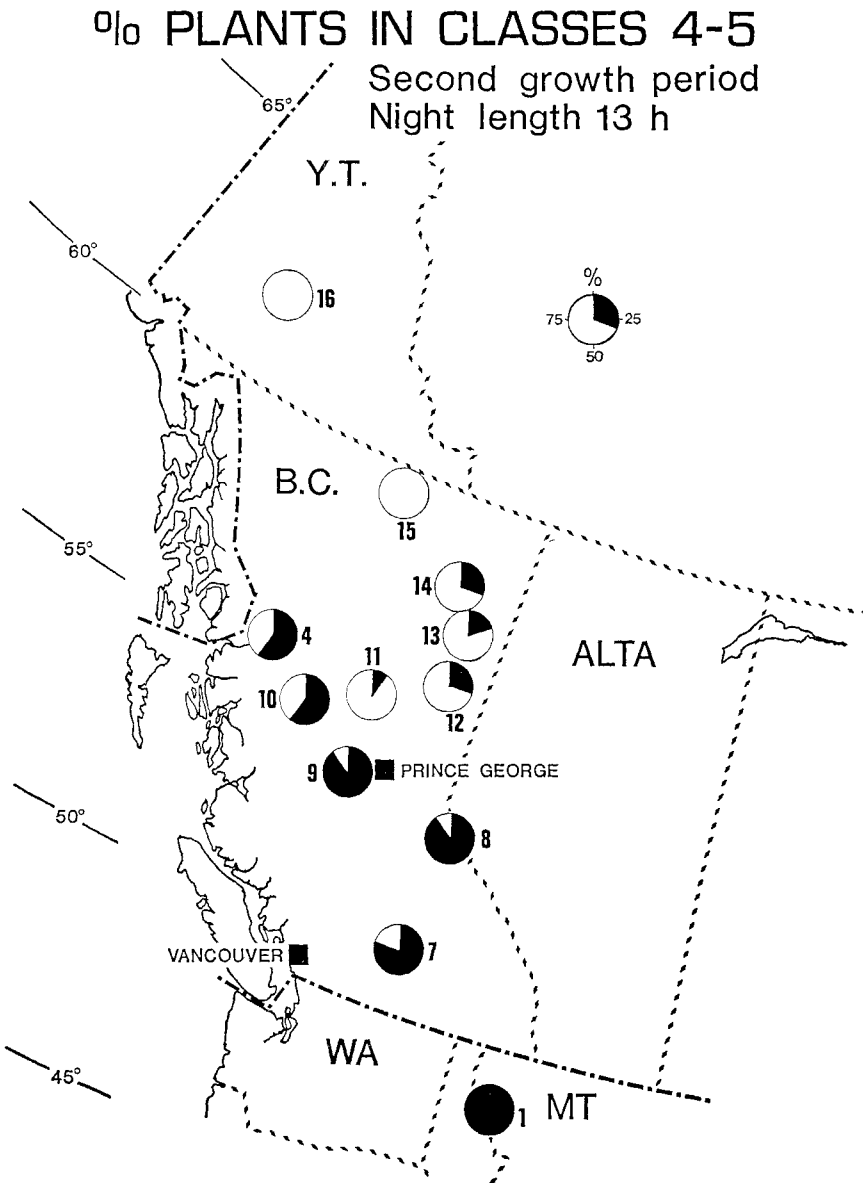


Figure 10. Experiment IV—second growth period. Percentage of plants in classes 4—5 (n = 10) in populations tested by freezing to -10°C during their second growth period. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. The treatment preceding the freezing test consisted of day/night temperatures $25/5^{\circ}\text{C}$ and a night length of 13 hours.

Table 4. Experiments IV and V, field trials 18—19, 30—31. The equations of the regressions and multiple correlation coefficients (R^2) from the stepwise regression analyses calculated for several characters connected with hardiness with five independent variables—latitude, latitude², altitude, altitude² and latitude×altitude. Only the variables showing significance are included in the equations.

Dependent variable	Growth period	Treat-ment night (h)	Equation	R^2	Shown in Figure
mean	1	12	$y = -44.24 + 1.9805 \text{ lat.} - 0.0201 \text{ lat.}^2$	0.85	
frost	1	13	$y = 11.76 - 0.0029 \text{ lat.}^2 - 0.0140 \text{ alt.}^2$	0.91	
damage	1	14	$y = 9.66 - 0.1573 \text{ lat.}$	0.74	
	2	13	$y = 8.90 - 0.0020 \text{ lat.}^2$	0.67	
percentage of plants in classes 4 and 5	1	12	$y = 376.43 - 0.0912 \text{ lat.}^2 - 0.4512 \text{ alt.}^2$	0.78	11
	1	13	$y = 1155.95 - 35.3599 \text{ lat.} - 0.0546 \text{ lat.} \cdot \text{alt.} + 0.2746 \text{ lat.}^2$	0.92	13
	2	13	$y = 273.86 - 0.0740 \text{ lat.}^2$	0.73	15
percentage of plants in classes 3 to 5	1	12	$y = -1310.54 - 0.5683 \text{ lat.}^2 + 56.7034 \text{ lat.}$	0.92	12
	1	13	$y = 529.96 - 8.4358 \text{ lat.} - 0.4202 \text{ alt.}^2$	0.88	14
	2	13	$y = 239.04 - 0.0566 \text{ lat.}^2$	0.71	16
percentage of plants with an ordinary growth after freezing	1	13	$y = -248.34 + 5.7262 \text{ lat.}$	0.80	18
	1	14	$y = -1146.61 + 42.4788 \text{ lat.} - 0.3613 \text{ lat.}^2$	0.94	
percentage mortality	trial:18		$y = 210.33 - 0.0494 \text{ lat.}^2 - 0.1564 \text{ alt.}^2$	0.78	28
	19		$y = 208.59 - 0.0454 \text{ lat.}^2 - 0.1336 \text{ alt.}^2$	0.74	28
	30		$y = 1829.01 - 57.3412 \text{ lat.} - 0.0470 \text{ lat.} \cdot \text{alt.} + 0.4589 \text{ lat.}^2$	0.93	28
	31		$y = 390.02 - 5.7055 \text{ lat.} - 0.0508 \text{ lat.} \cdot \text{alt.}$	0.83	28

differences in latitude and altitude.

The results of the regression analyses are illustrated in Figures 11—16. Only the independent variables showing significance were considered when drawing the diagrams. There is a good agreement between all the figures. In all cases the latitude plays an important role as indicated by a steep declination of the curves. Only in a minority of the regressions did the altitude exert a significant influence. However, it must be noted that the altitude did not result in any considerable improvement of the R^2 -values of the regressions. It must also be remembered that the experiments were not designed in such a way that the influence of altitude on the development

of hardiness could be tested as accurately as the influence of latitude. The results from the populations at latitude 55—56°N suggest that the altitude at a certain latitude is of significance for the development of hardiness (cf. Figures 7—9). The columns in Figure 17 based on data from the second growth period constitute a good example of this.

It is worth mentioning that a complete agreement (i.e. the same percentage of injured plants) between the results at 13 h night length during the first and second growth periods is not expected. The pretreatment of the plants tested during the second growth period may cause a shift of the development of hardiness towards a

Percentage of plants in classes 4-5

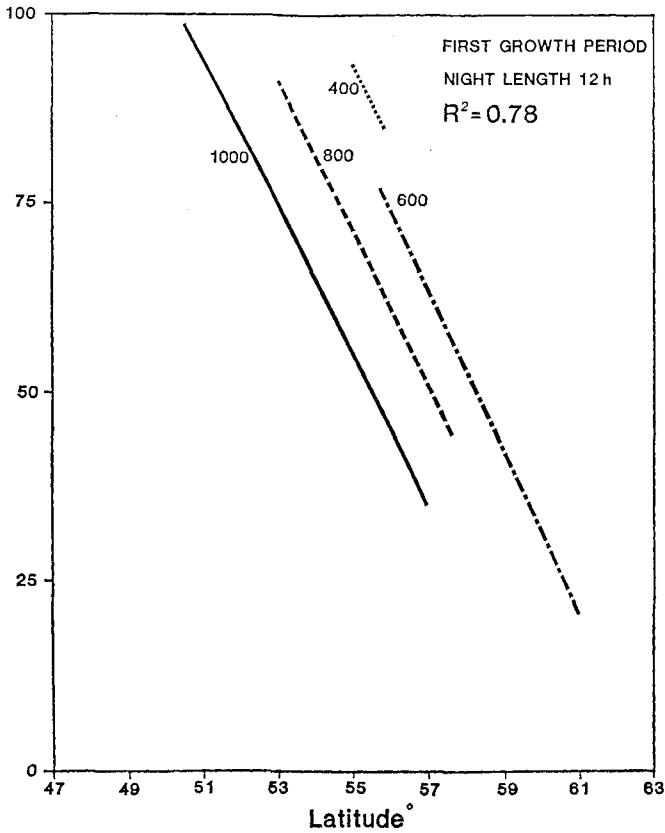


Figure 11. Experiment V. Regression of percentage of plants in classes 4-5 ($n = 15$) on latitude at constant altitudes. Plants in the first growth period were frozen to -10°C after one week of treatment with 12 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equation of the regression in Table 4.

Percentage of plants in classes 3-5

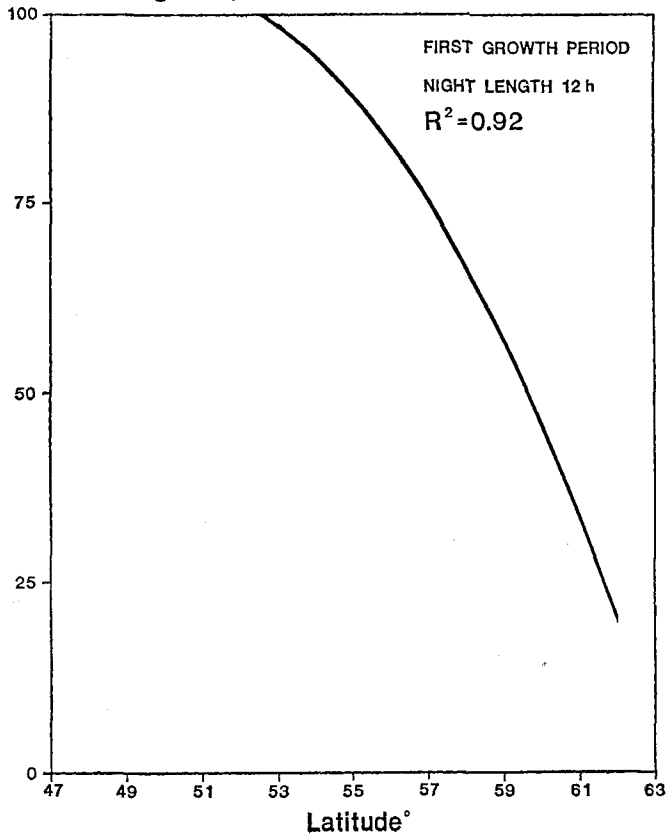


Figure 12. Experiment V. Regression of percentage of plants in classes 3-5 ($n = 15$) on latitude. Plants in the first growth period were frozen to -10°C after one week of treatment with 12 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equation of the regression in Table 4.

Percentage of plants in classes 4-5

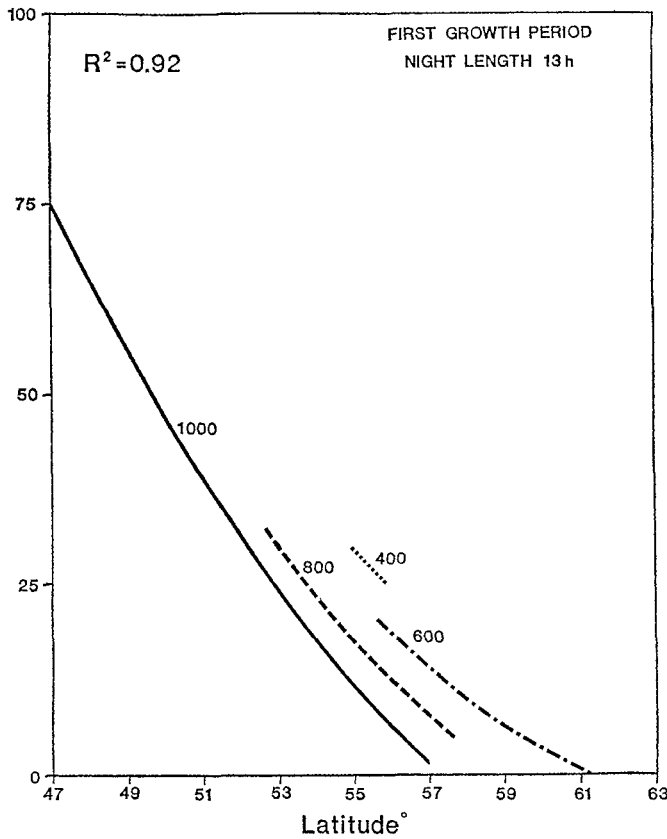


Figure 13. Experiment IV. Regression of percentage of plants in classes 4-5 ($n = 15$) on latitude at constant altitudes. Plants in the first growth period were frozen to -10°C after one week of treatment with 13 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equation of the regression in Table 4.

Percentage of plants in classes 3-5

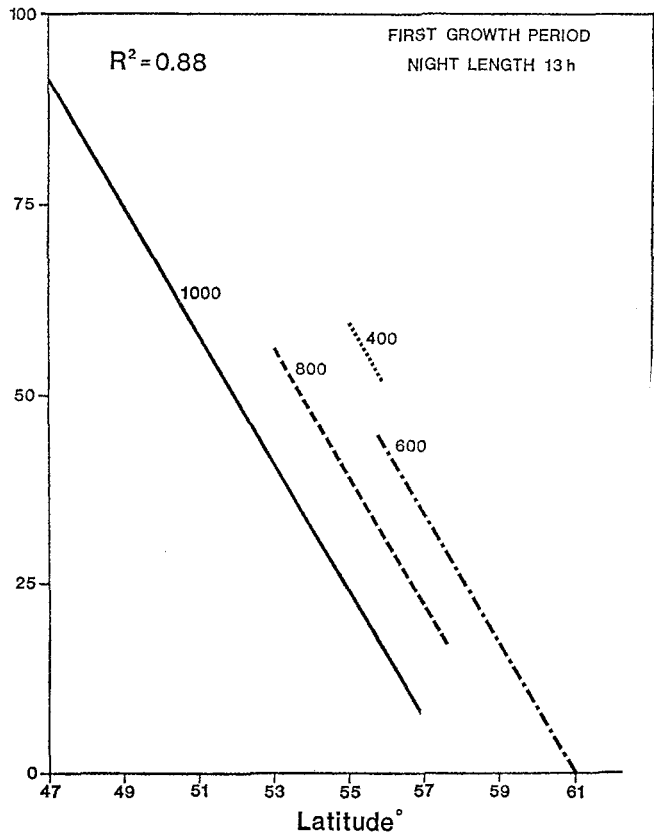


Figure 14. Experiment IV. Regression of percentage of plants in classes 3-5 ($n = 15$) on latitude at constant altitudes. Plants in the first growth period were frozen to -10°C after one week of treatment with 13 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equation of the regression in Table 4.

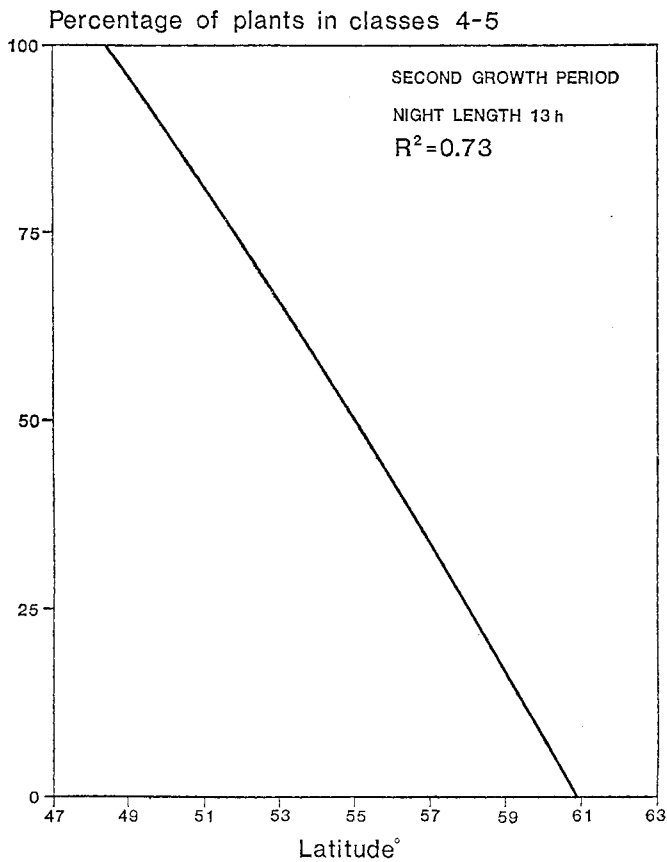


Figure 15. Experiment IV—second growth period. Regression of percentage of plants in classes 4—5 ($n=10$) on latitude. Plants in the second growth period were frozen to -10°C after one week of treatment with 13 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equation of the regression in Table 4.

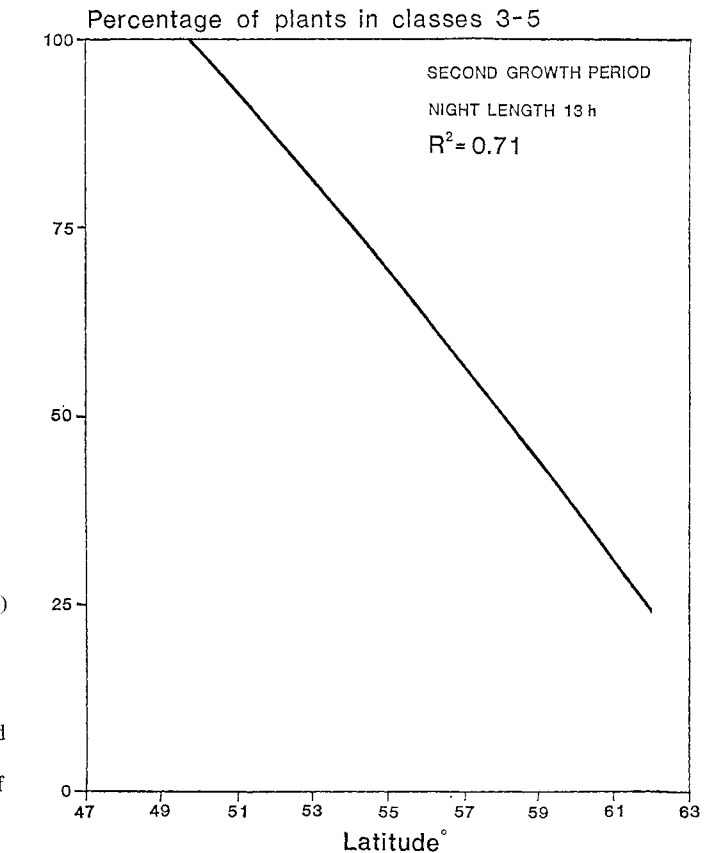


Figure 16. Experiment IV—second growth period. Regression of percentage of plants in classes 3—5 ($n=10$) on latitude. Plants in the second growth period were frozen to -10°C after one week of treatment with 13 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equation of the regression in Table 4.

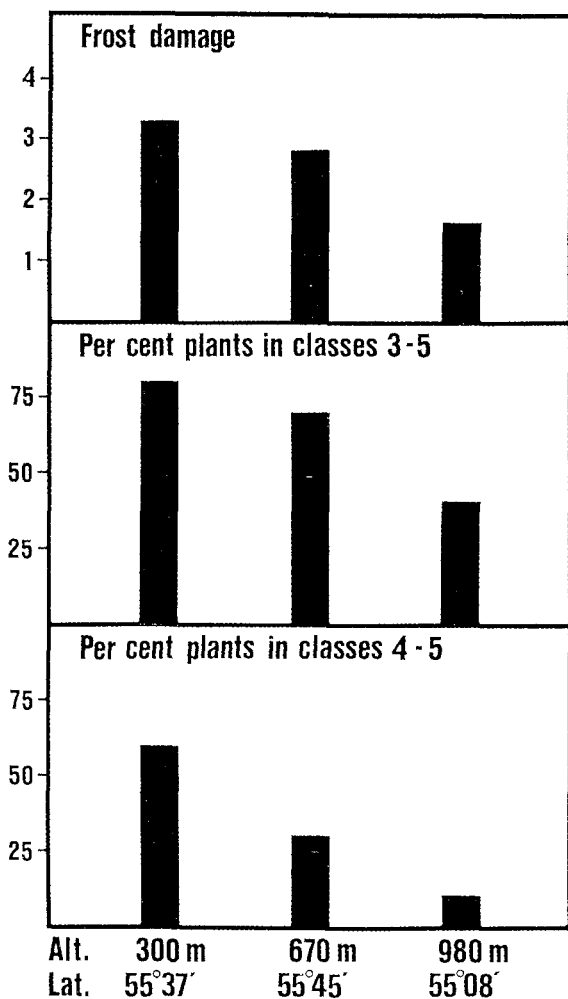


Figure 17. Experiment IV—second growth period. Frost damage ($n = 10$) and percentages of plants in classes 3—5 as well as 4—5 in plants frozen to -10°C after treatment with 13 h night. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Influence of altitude is illustrated by means of three populations (Nos. 4, 12 and 11 in Table 1), originating from latitude 55° .

shorter or longer night length. Alternatively, the hardiness at a certain photoperiod may not be the same during the first and second growth periods even if the pretreatment could be ruled out as an influential agent. On the other hand the ranking of the populations from one treatment to another was not changed (see also Table 5).

Based on the percentage of un lignified cells in twelve provenances of *P. contorta*, Hagner calculated a regression (Hagner 1970b, Figure 10) which, in the latitude range 52° — 60° , agrees well with our data on the percentage of plants in classes 3—5 at a night length of 12 h. In Hagner's

regression $(\text{lat}-50)^2$ was the only significant independent variable.

3.2.3 Plant growth after freeze testing

Some examples of the influence of photoperiod and origin (latitude and altitude) on the development of hardiness are illustrated by the photographs in Plates 1—6. To illustrate the influence of latitude the two most extreme populations and a centre located one were selected.

The plants in the photographs reveal that the growth following exposure to -10°C varied considerably both between and within populations. This character must

treated with care since the photoperiodic treatment preceding the freezing test influences the flushing of the northerly and southerly populations in different ways.

The latitudinal and altitudinal influence on the percentage of plants with a normal growth following freezing were tested. Plants with leaders showing deviations from the ordinary pattern of growth were not included in that percentage. A stepwise regression analysis was carried out with the same five independent variables as used in previously described regressions. Once more, latitude was shown to be the most important variable. The regression calculated is illustrated in Figure 18.

3.2.4 Some examples from literature of clinal variation in conifers

The clinal variation observed in the present investigation agrees well with earlier results

concerning survival of *P. contorta* (Dietrichson 1970, Hagner & Fahlroth 1974, Lindgren et al. 1976 and 1980, Rosvall 1980). Actually, in tree species from the temperate zone there is generally a clinal variation for characters of adaptive significance (cf. Langlet 1936 and 1959, Eiche 1966, Holzer 1966, Dormling 1973 and 1979, Larsen 1978a and c, Eriksson et al. 1980). Further references to Northern America tree species will be found in Morgenstern (1978). The absence of a clinal variation for lignification reported by Hagner (1980) was explained by attacks of *Fusarium* root rot on the seedlings. On the other hand several other characters studied by Hagner showed a clinal variation.

A clinal variation was also observed for isozymes and terpenes in *P. contorta* (Yeh & Layton 1979, Forrest 1979). Ekberg et al. (1970) reported a clinal variation with re-

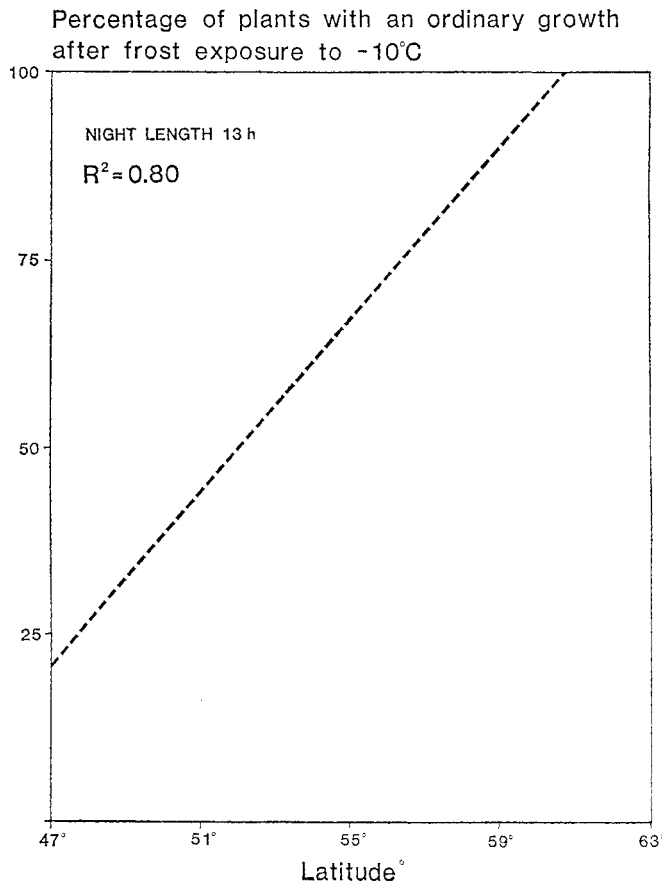


Figure 18. Experiment IV. Regression of percentage of plants with an ordinary growth ($n = 15$) 6 weeks after freezing to -10°C on latitude. Plants were tested during their first growth period after treatment with 13 h night. Equation of the regression in Table 4. For definition of ordinary growth see the text.

Table 5. Experiments IV and V. Correlation coefficients showing the relationship between different ways of expressing the extent and severity of frost damage during first and second growth period after different photoperiodic treatments. The levels of significance: *** - 0.05 %, ** - 0.5 %, * - 2.5 %.

Type of treatment and material	12 h 1st growth period			13 h 1st growth period			13 h 2nd growth period		
	Character	mean frost damage	% plants in classes 4-5	% plants in classes 3-5	mean frost damage	% plants in classes 4-5	% plants in classes 3-5	mean frost damage	% plants in classes 4-5
12 h 1st growth period	% plants in classes 4-5	0.943 ***							
	% plants in classes 3-5	0.978 ***	0.903 ***						
	mean frost damage	0.898 ***	0.865 ***	0.874 ***					
13 h 1st growth period	% plants in classes 4-5	0.679 *	0.642 *	0.667 *	0.911 ***				
	% plants in classes 3-5	0.847 ***	0.832 **	0.820 **	0.991 ***	0.918 ***			
	mean frost damage	0.934 ***	0.915 ***	0.889 ***	0.899 ***	0.721 **	0.872 ***		
13 h 2nd growth period	% plants in classes 4-5	0.882 ***	0.885 ***	0.837 **	0.929 ***	0.801 **	0.912 ***	0.947 ***	
	% plants in classes 3-5	0.931 ***	0.921 ***	0.910 ***	0.907 ***	0.745 **	0.883 ***	0.985 ***	0.939 ***

spect to onset of meiosis in pollen mother cells. Even if we frequently observe a clinal variation it does not mean that there is no variation within a provenance (cf. Dietrichson 1970, Perry & Lotan 1978). On the contrary, physiographic barriers have caused development of great genetic variation within limited geographic areas as stressed by Illingworth (1976). Future work in climate chambers ought therefore to be directed towards studies on the genetic variation between and within populations

from a geographically limited area. Such a study of populations along a mountain slope ought to give information on the evolution of hardiness.

3.2.5 Comparison between frost damage studied at different photoperiods and expressed in different ways

In Table 5 correlation coefficients are listed showing the degree of correlation between different ways of expressing the extent of

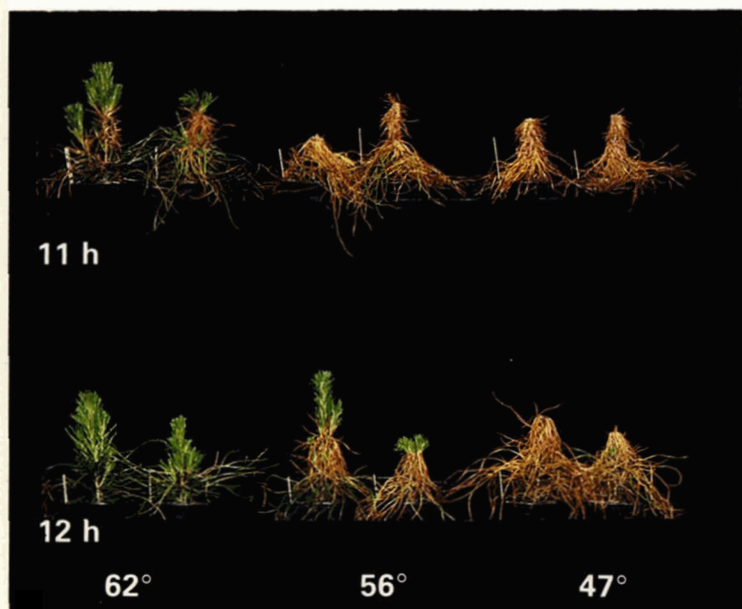


Plate 1.

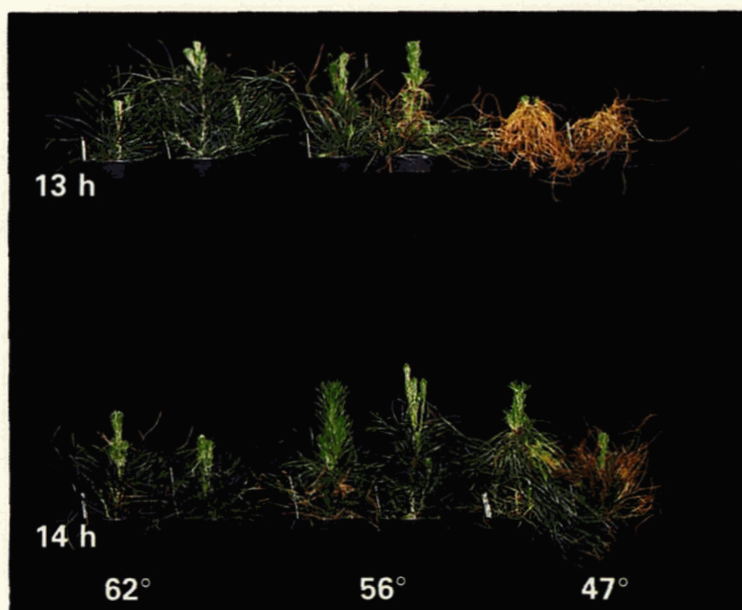


Plate 2.

Plates 1 and 2.

Experiments IV and V. Two plants from each of three populations (Nos. 16, 12 and 1) representing the range of latitudes occurring in the material studied, tested by freezing to -10°C . The treatment preceding the freezing tests consisted of gradually prolonged nights at day/night temperatures $25/5^{\circ}\text{C}$. The freezing tests were performed after weeks with 11, 12, 13 and 14 hours night respectively and five weeks later the damage by frost was documented by photographing.

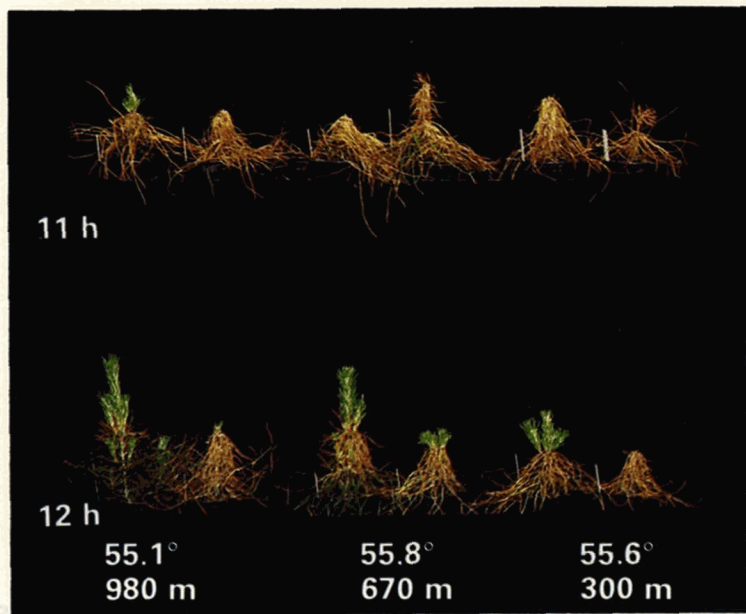


Plate 3.

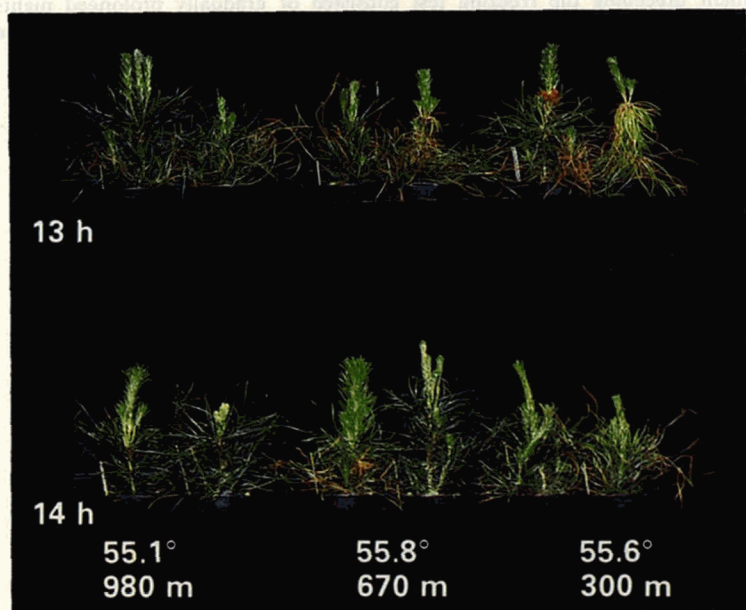


Plate 4.

Plates 3 and 4.

Experiments IV and V. Two plants from each of three populations (Nos. 11, 12 and 4) originating from the same latitude (55.1–55.8°) but varying altitudes (980, 670 and 300 m respectively) tested by freezing to -10°C . The treatment preceding the freezing tests consisted of gradually prolonged nights at day/night temperatures $25/5^{\circ}\text{C}$. The freezing tests were performed after weeks with 11, 12, 13 and 14 hours night respectively. Photos taken five weeks after freezing.

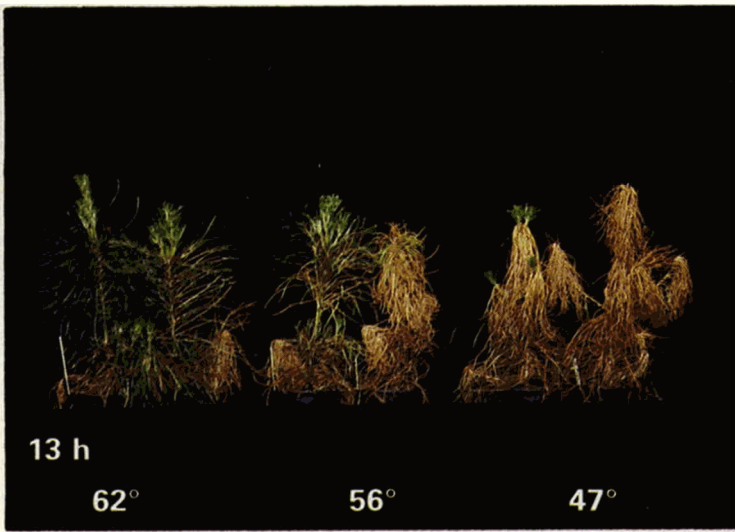


Plate 5.

Experiment IV—second growth period. Two plants from each of three populations (Nos. 16, 12 and 1) representing the range of latitudes occurring in the material studied, tested by freezing to -10°C . The treatment preceding the freezing test consisted of gradually prolonged nights at day/night temperatures $25/5^{\circ}\text{C}$. The freezing was performed after one week with 13 hours night and the photo was taken five weeks later.

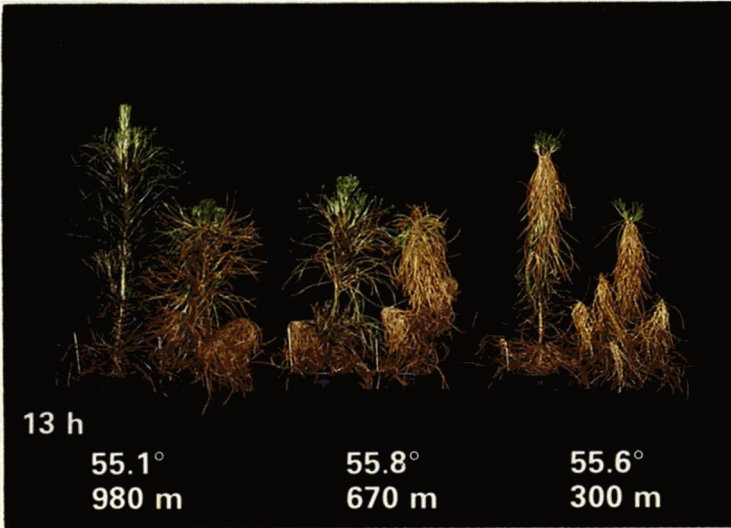


Plate 6.

Experiment IV—second growth period. Two plants from each of three populations (Nos. 11, 12 and 4) originating from the same latitude (55.1° — 55.8°) but varying altitudes (980, 670 and 300 m respectively) tested by freezing to -10°C . The treatment preceding the freezing test consisted of gradually prolonged nights at day/night temperatures $25/5^{\circ}\text{C}$. The freezing test was performed after one week with 13 hours night and the photo was taken five weeks later.

damage at varying night lengths. As may be seen, there is good agreement between mean frost damage score, the percentage of plants in classes 3—5 and the percentage of plants in classes 4—5 within each of the treatments. The correlations showed that the relatively convenient way of expressing the amount and degree of damage as the mean frost damage constitutes a good parameter for describing hardiness of different populations.

A highly significant correlation exists also between the results from plants tested at 12 and 13 h nights respectively. A good agreement between data from the first and second growth period was also noted.

Summary. A great genetic variation in frost hardiness was noted among the populations studied in the present investigation. Their origin varied between latitudes 46° and 63°. A strong latitudinal influence on the frost hardiness attained at a certain photoperiodic treatment was disclosed by regression analysis. Altitude also seems to influence frost hardiness.

By the present experimental technique a good separation of genetic entries with respect to frost hardiness is possible following exposure to low temperatures at night lengths of 12 and 13 hours.

3.3 Characters correlated with hardiness

In experiment I, a pilot test of the photo- and thermoperiodic response of 16 characters was carried out. The results were plotted in diagrams of the type illustrated in Figures 19—20. A separate regression analysis for the two populations was carried out for each of the traits tested. Night length, temperature during daylight, temperature difference between day and night, and their squares and products were used as independent variables. The results are demonstrated in Table 6. As may be seen from this table, photoperiod, temperature difference between day and night and day temperature explained 0—94% of the variation of the individual characters.

Among the independent variables tested night length appeared most frequently in step 1 in the regression analysis.

Hellmers (1962) reported that the difference between day and night temperature was the critical temperature parameter for growth of *Pinus taeda*. Similar results were obtained for *Pinus sylvestris* by Dormling (1975). Hellmers & Rook (1973) on the other hand reported that a low night temperature rather than a high one promoted a faster growth of *Pinus radiata* seedlings.

The low R²-values of the dry matter content need a special comment. This test was not designed to study development of frost hardiness since the night lengths 4—8 h were *a priori* expected to be too short to influence dry matter content to the full extent. This is supported by the fact that the R²-value of the southerly population was lower than the value of the northerly population. Thus, the night length is expected to exert its influence at shorter night lengths in the northerly population than in the southerly one.

3.3.1 Correlations at the population level

In experiments IV—V we concentrated our studies on the correlation between hardiness and specific traits to three characters that seemed to be governed by the photoperiod: lengthening of secondary needles, dry matter content, and anthocyanin colour.

Lengthening of secondary needles

The advantage of using lengthening of secondary needles is that several measurements of this character as well as the freezing test may be carried out on the same plant.

Some data from experiments II—III were published previously (cf. Dormling et al. 1977a, Figure 4, and Dormling et al. 1977b, Figure 5). There it was shown how the growth of secondary needles decreased with increasing length of night. This tendency was more pronounced at a night temper-

St Regis, MT 47°22'

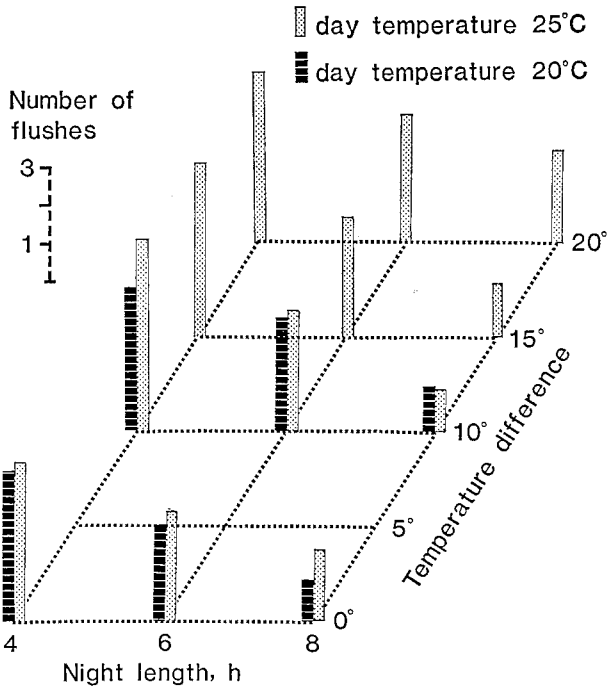


Figure 19. Experiment I. Relationship between number of flushes on the one hand and night length in hours as well as the difference between day and night temperature in °C at two temperature levels on the other hand. Assessment after 26 weeks of population No. 1, St. Regis, Montana of *P. contorta* studied in this experiment.

Yakutat, AK 59°30'

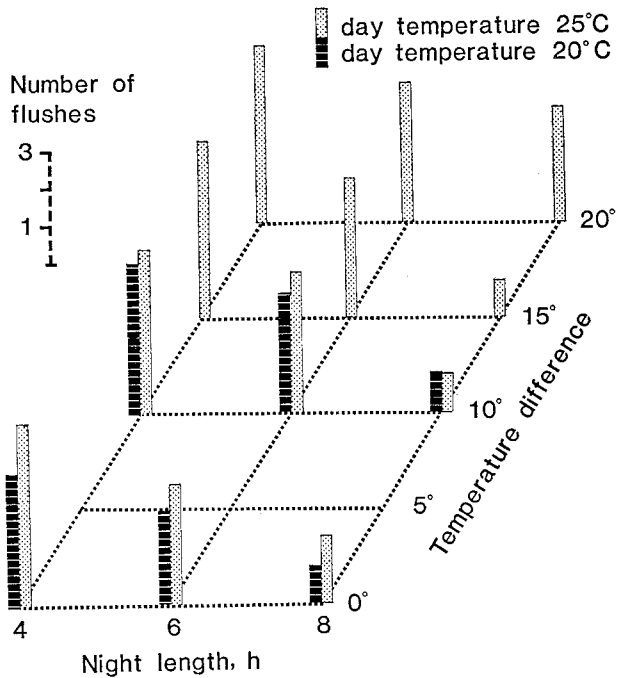


Figure 20. Experiment I. Relationship between number of flushes on the one hand and night length in hours as well as the difference between day and night temperature in °C at two temperature levels on the other hand. Assessment after 26 weeks of population No. 2, Yakutat, Alaska of *P. contorta* studied in this experiment.

ture of +3°C than at +15°C. The correlation between the growth of secondary needles the week before freezing and mean frost damage was strong ($r=0.91^{***}$, significant at the 0.05 per cent level).

The regressions of frost damage (=the percentage of plants in classes 4–5) on growth of secondary needles calculated for the 12 populations in experiments IV and V are illustrated in Figure 21. As may be seen in this figure the individual values fit fairly well to the regression lines for the night lengths 12–14 h. With the freeze testing of plants during the second growth period a strong correlation was also noted (cf. Figure 22). This means that recording the growth of secondary needles at proper night lengths constitutes a good means of distinguishing hardy and non-hardy populations. The equations of the regressions discussed above as well as the ones regarding mean frost damage are listed in Table 7.

Dry matter content

In experiments II and III the dry matter content of the uppermost three centimetres of the plant top was shown to be strongly correlated with the hardness of the seedlings (cf. Figure 5 in Dormling et al. 1977a, Figure 6 in Dormling et al. 1977b, and Figure 4 in Jonsson et al. 1979).

The relationships between the percentage of plants in classes 4–5 and the dry matter content based on data from experiments IV–V are illustrated in Figure 23. The corresponding equations are presented in Table 7. Figure 23 resembles a reflected image of Figure 21, which is due to the fact that dry matter content and percentage of injured plants are negatively correlated with each other. The fit of the values to the regression lines for night lengths 12 and 13 h is as good as for lengthening of the secondary needles. However, the destructive character of the assessment of

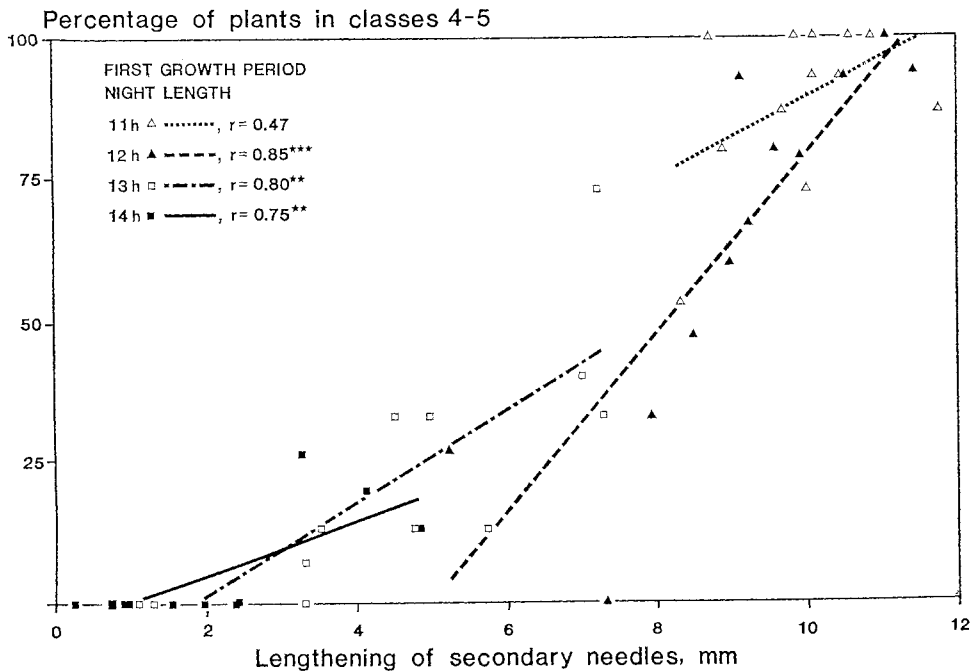


Figure 21. Experiments IV and V. Regressions of percentage of plants in classes 4–5 ($n=15$) after freezing to -10°C on lengthening of secondary needles ($n=30$) in the week preceding the freezing test. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equations of the regressions in Table 7, the levels of significance being: *** — 0.05%, ** — 0.5%, * — 2.5%.

Table 6. Experiment I. Characters studied in 26 week-old plants (dependent variables) of populations Nos. 1 and 2, as well as the importance of the independent variables as indicated by the sequence in which they enter the first steps of the regression analysis and by R²-values. Independent variables: 3 — day temperature; 4 — difference between day and night temperatures; 5 — photoperiod; 3², 4², 5², 3×4, 3×5, 4×5 and 3×4×5.

Popu- lation	Dependent variable	Independent variables				R ²
		Step				
		1	2	3	4	
1	1 Plant height 1 = from pot edge to top of primary needles, mm	5	4×5	4 ²		0.72
2		5	5 ²	3×4×5		0.71
1	2 Plant height 2 = from pot edge to top of top bud, mm	4	4 ²			0.55
2		—	—			0
1	3 Needle length = length of longest secondary needle, mm	5	5 ²			0.83
2		5	5 ²			0.57
1	4 Diameter of stem base, mm	5 ²	3×5			0.63
2		5 ²	3×5			0.58
1	5 Dry weight, stem + needles, g	5	—			0.51
2		5 ²	—			0.58
1	6 Dry matter, percentage	4	—			0.22
2		4×5	3			0.48
1	7 Stem-needle relation	3×5	—			0.29
2		—	—			0
1	8 Plant height 3 = from cotyledons to base of top bud, mm	4	4 ²			0.48
2		3×4×5	—			0.23
1	9 Plant height 4 = from cotyledons to top of top bud, mm	4	4 ²			0.52
2		3×4×5	—			0.23
1	10 Length of top bud, mm	—				0
2		—				0
1	11 Height of first flush from cotelydons, mm	5	—			0.73
2		5	5 ²			0.80
1	12 Number of flushes	5	3×5			0.94
2		5	3×5			0.88
1	13 Number of primary needles on main stem	5	—			0.54
2		5	5 ²			0.62
1	14 Length of the part of main stem with primary needles, mm	5	4	4 ²	—	0.61
2		5	5 ²	3×4×5	4 ²	0.80
1	15 Needle frequency = 14:13	3×5				0.22
2		—				0
1	16 Number of lateral shoots	5	4 ²			0.85
2		3×5	3 ²			0.68

Population No. 1: St. Regis, Montana.
Population No. 2: Yakutat, Alaska.

Table 7. A compilation of the regressions discussed in chapter 3.3; a=linear regressions, b=stepwise regression. The correlation (*r*) and multiple correlation (*R*²) coefficients are given. Levels of significance: *** 0.05 %, ** 0.5 %, * 2.5 %.

Dependent variable y	Independent variable x	Growth period	Treatment (h night)	Equation	a) <i>r</i> b) <i>R</i> ²	Shown in Figure
a) mean frost damage	lengthening of secondary needles	1	11	$y = 0.18x + 2.64$	0.45	
			12	$y = 0.56x - 1.49$	0.93***	
		2	13	$y = 0.51x - 0.29$	0.93***	
			14	$y = 0.44x + 0.09$	0.90***	
	percentage of plants in classes 4 and 5	1	11	$y = 0.46x + 1.33$	0.86***	22
			13	$y = 7.00x + 19.28$	0.47	21
		2	12	$y = 15.83x - 79.06$	0.85***	21
			13	$y = 8.34x - 15.87$	0.80**	21
	mean frost damage	1	11	$y = 4.90x - 4.84$	0.75**	21
			14	$y = 17.11x - 8.82$	0.90***	22
		2	13	$y = 9.73 - 0.24x$	-0.65*	
			12	$y = 19.37 - 0.66x$	-0.96***	
percentage of plants in classes 4 and 5	1	11	$y = 16.11 - 0.55x$	-0.85***		
		13	$y = 9.79 - 0.32x$	-0.78**		
	2	14	$y = 19.29 - 0.61x$	-0.76**		
		13	$y = 256.43 - 7.48x$	-0.55	23	
mean frost damage	1	11	$y = 539.75 - 20.02x$	-0.94***	23	
		12	$y = 226.46 - 8.08x$	-0.65*	23	
	2	13	$y = 74.41 - 2.55x$	-0.46	23	
		14	$y = 649.24 - 22.23x$	-0.78**	25	
percentage of plants in classes 4 and 5	1	11	$y = 11.79 - 0.34x$	-0.88***		
		12	$y = 20.80 - 0.74x$	-0.85***		
	2	13	$y = 27.58 - 1.02x$	-0.80**		
		13	$y = 305.35 - 10.04x$	-0.70*	24	
mean frost damage	1	11	$y = 562.45 - 21.45x$	-0.80**	24	
		12	$y = 929.33 - 36.28x$	-0.80**	25	
	2	13	$y = 929.33 - 36.28x$	-0.80**	25	
		13	$y = 929.33 - 36.28x$	-0.80**	25	
dry matter content in apical shoot	1	11	$y = 6.20 - 3.87x$	-0.86***		
		14	$y = 3.52 - 2.33x$	-0.83***		
	2	13	$y = 5.90 - 2.80x$	-0.79**		
		13	$y = 1.02x + 0.02$	0.84***		
b) intensity of anthocyanin colour	1	11	$y = 1.09x - 1.34$	0.93***		
		12	$y = 1.09x - 1.34$	0.93***		
	2	13	$y = 0.97x + 2.25$	0.88***		
		14	$y = 0.80x + 7.83$	0.74**		
b) intensity of anthocyanin colour	1	11	$y = 1.33x - 5.50$	0.88***		
		13	$y = 1.33x - 5.50$	0.88***		
	2	13	$y = 1.33x - 5.50$	0.88***		
		13	$y = 1.33x - 5.50$	0.88***		
b) intensity of anthocyanin colour	1	16	$y = -2.47 + 0.0575 \text{ lat.} + 0.0009 \text{ lat. alt.}$	0.77	26	
		16	$y = -2.47 + 0.0575 \text{ lat.} + 0.0009 \text{ lat. alt.}$	0.77	26	
	2	16	$y = -2.47 + 0.0575 \text{ lat.} + 0.0009 \text{ lat. alt.}$	0.77	26	
		16	$y = -2.47 + 0.0575 \text{ lat.} + 0.0009 \text{ lat. alt.}$	0.77	26	

Figure 22. Experiment IV—second growth period.

Regressions of frost damage as well as percentage of plants in classes 4–5 ($n = 10$) after freezing to -10°C on lengthening of secondary needles ($n = 20$) in the week preceding the freezing test. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equations of the regressions in Table 7, the levels of significance as in Figure 21.

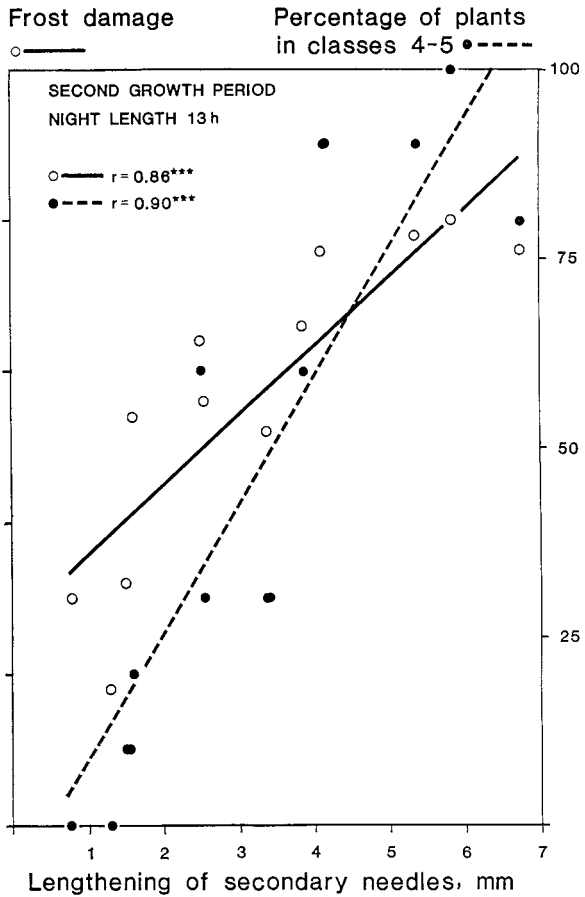
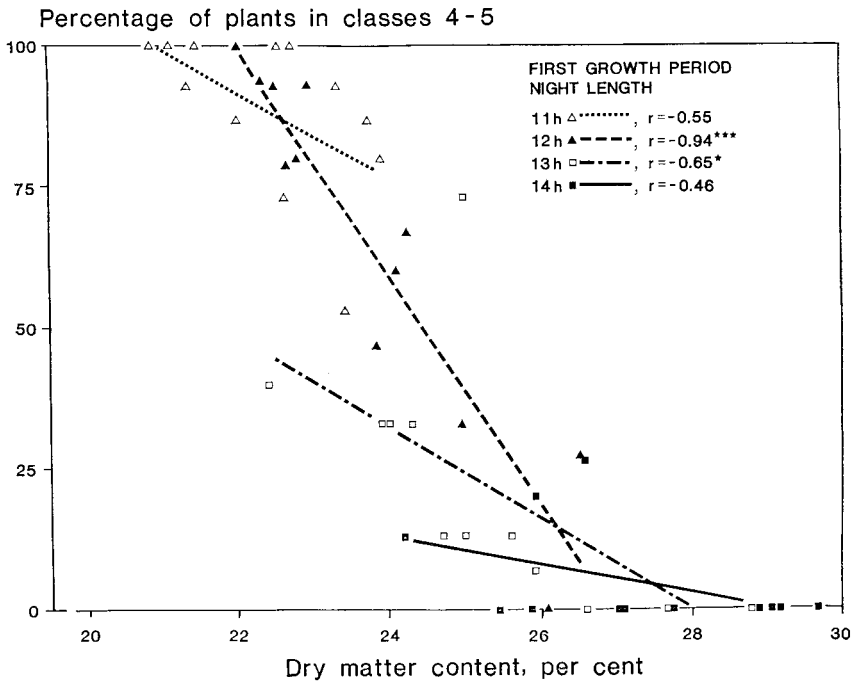


Figure 23 (below).

Experiments IV and V.

Regressions of percentage of plants in classes 4–5 ($n = 15$) after freezing to -10°C on dry matter content ($n = 10$) estimated simultaneously in apical shoots of plants treated with the same photoperiod as plants intended for freezing. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equations of the regressions in Table 7, the levels of significance being as in Figure 21.



the dry matter content does not allow a testing of the same plant both with respect to frost damage and dry matter content. This drawback may be circumvented if the dry matter content could be assessed on lateral shoots.

A study of the correlation between the dry matter content in the top and lateral shoots was carried out on the plants at the end of the periods with night lengths 11, 12, 13, and 14 h respectively. As may be seen in Table 7, the correlation coefficients were significant at the 0.05 and 0.5% levels, respectively.

Therefore, in experiment V the correlation between frost damage (=the percentage of plants in classes 4—5) and dry matter content of lateral shoots of plants intended for freeze testing was determined. As seen from Figure 24 the fit to the regression line was fairly good both at night lengths 11 h and 12 h. The correla-

tion obtained from the material tested during the second growth period also showed a fairly good fit to the regression (cf. Figure 25 and Table 7). In experiments II—III, two populations of *Pinus sylvestris* were included. A comparison of the dry matter contents responsible for development of a satisfactory hardiness in *P. contorta* and *P. sylvestris* was carried out (cf. Dormling et al. 1977a and b). The result of this comparison suggested that a somewhat higher dry matter content was needed in *P. contorta* to attain the same hardiness as in *P. sylvestris*. This confirms earlier results by other investigators (Langlet 1936, 1938, Dietrichson 1970). This caused Dietrichson (1970) to state that "the dry matter content must be used to distinguish genetic entries only within a species with respect to hardiness" (free translation from Norwegian), a statement we support.

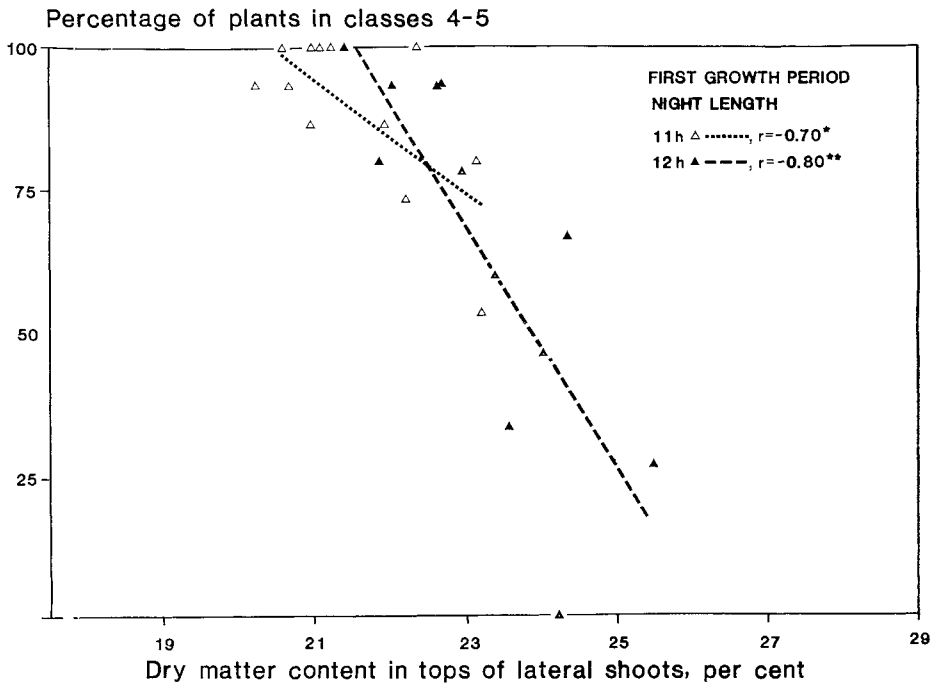


Figure 24. Experiment V. Regressions of percentage of plants in classes 4—5 after freezing to -10°C ($n = 15$) on dry matter content estimated in lateral shoots of tested plants immediately before freezing. The photoperiods applied were 11 and 12 hours night respectively. The scoring comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equations of the regressions in Table 7, the levels of significance being as in Figure 21.

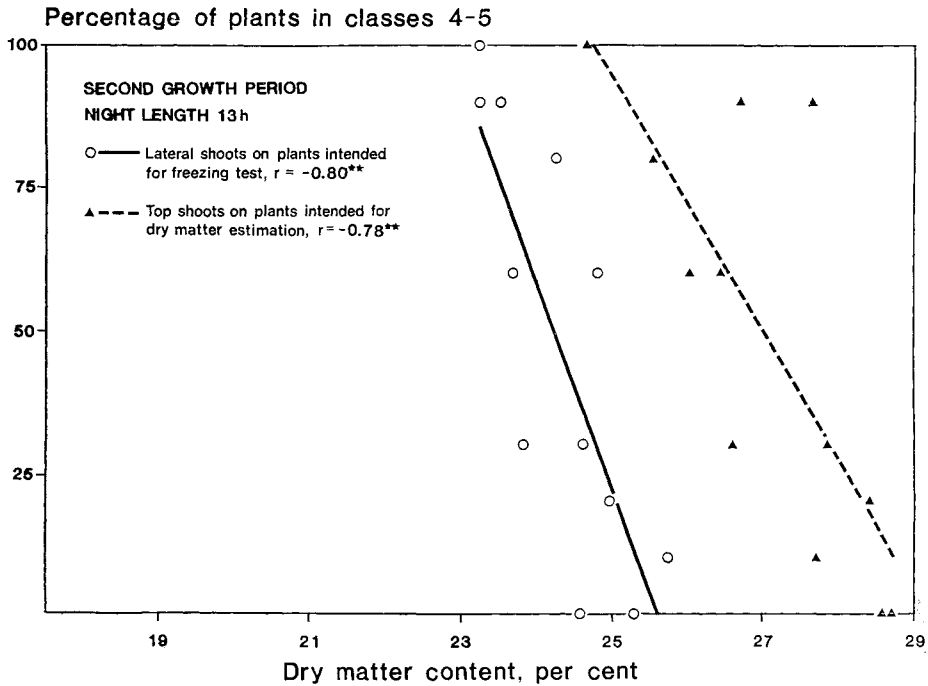


Figure 25. Experiment IV—second growth period. Regressions of percentages of plants in classes 4–5 after freezing to -10°C ($n=10$) on dry matter content estimated a) in lateral shoots of the plants intended for the freezing test ($n=10$), b) in apical shoots of plants treated with the same photoperiod as the plants intended for the freezing test ($n=10$). The scoring comprised 6 classes: 0=no damage, 5=dead apical bud. Equations of the regressions in Table 7, the levels of significance being as in Figure 21.

Anthocyanin colour

The intensity of anthocyanin colour during the hardening process is another character that seems to be strongly influenced by the origin of the populations. Four classes, 0–3, were distinguished, where 0 represents no visible traces of anthocyanin colour and 3 represents intensive colour. The regressions in Figure 26 illustrate how this character depends on latitude and altitude of the populations investigated. The more northern the origin, the more readily the colour is developed. The equation of the regression is presented in Table 7.

The strong correlations between mean values of anthocyanin colour and mean frost damage after freezing at night lengths 13 h and 14 h may be seen from Figure 27 and Table 7. A strong correlation was also noted for the populations tested at a night

length of 13 h during the second growth period (see Table 7).

3.3.2 *Correlations at the individual level*

The existence of a strong correlation at the individual level between frost hardiness and a specific trait is of great value for clonal forestry. Thus, outstanding clones might be identified in early tests and then mass propagated.

There are difficulties in determining such a correlation since the assessment of frost damage had to be done by discrete figures 0, 1, ..., 5 in individual plants whereas continuous figures could be used at the population level. The limited number of plants that could be tested was another constraint to the possibilities of determining this correlation. Therefore, too far-reaching

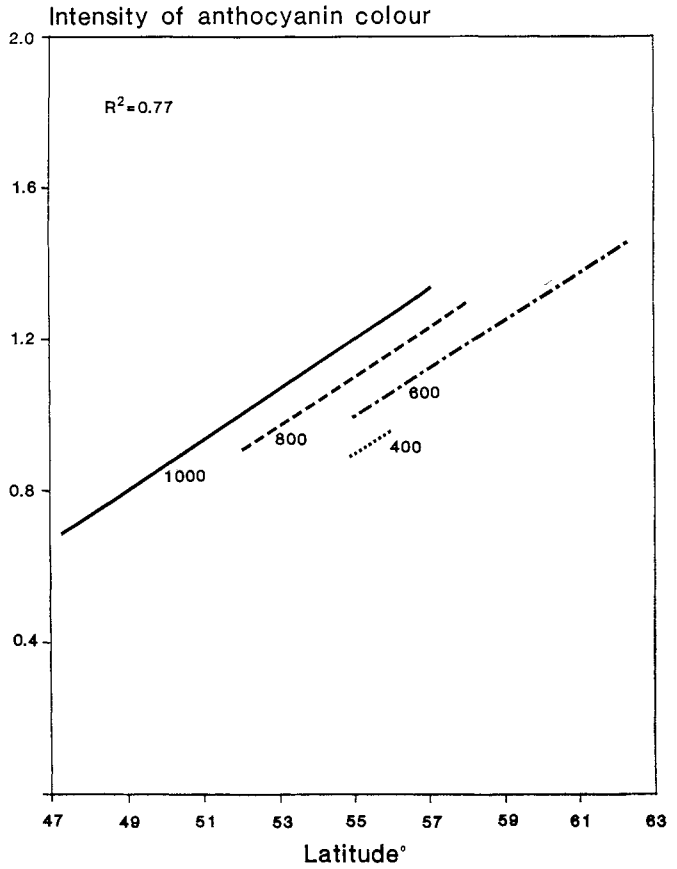


Figure 26. Experiment IV. Regression of the intensity of anthocyanin colour on latitude at four altitudes. The intensity of anthocyanin colour was classified on a scale 0–3 after 3 weeks of hardening with long nights (16 h) and low temperatures (15/5°C, cf. Table 3). 0 = no visible violet colour, 3 = very intense colour. Equation of the regression in Table 7.

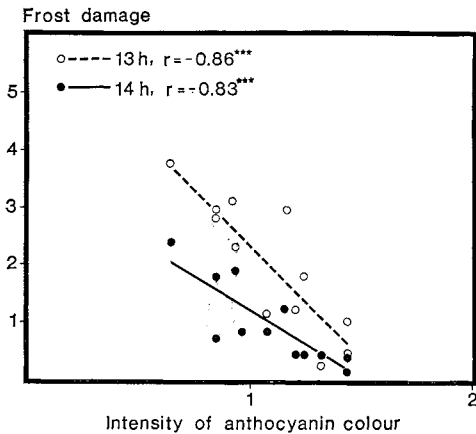


Figure 27. Experiment IV. Regressions of frost damage ($n=15$) after freezing to -10°C following a treatment with 13 and 14 hours night on the intensity of anthocyanin colour ($n=25$) classified on a scale 0–3 under the period of hardening. 0 = no visible violet colour, 3 = very intense colour. The scoring of frost damage comprised 6 classes: 0 = no damage, 5 = dead apical bud. Equations of the regressions in Table 7, the levels of significance as in Figure 21.

conclusions must not be based on the calculations, which were carried out separately for the twelve populations. It may suffice to say, that approximately half of the correlations between frost damage and dry matter content were significant at the 2.5 or 0.5 per cent level. Once more it must be stressed that one hardly expects any correlations within the populations with a mean frost damage close to 0 or 5. The results must be regarded as rewarding and call for more extensive studies along these lines.

Summary. Strong correlations at the population level between frost damage and the following three traits were noted:

- lengthening of secondary needles,
- dry matter content of the apical or lateral shoots (uppermost 3 cm), and
- anthocyanin colour.

The correlations between frost damage and dry matter content at the individual level have to be studied in larger plant populations, but the results so far must be regarded as promising.

3.4 The agreement between field data and climate chamber data as regards genetic variation in hardiness

Forest tree breeding would benefit much from early testing if the characters used were good predictors of the future development of a certain genetic entry. In chapters 3.1 and 3.2 the strong influence of photoperiod on the development of the hardiness of seedlings was shown. The photoperiod exerts an influence on the rate of development of needle primordia also of older *P. contorta* plants according to Cannell & Willett (1975). It may be assumed, therefore, that the data regarding hardiness of seedlings could be used in practical tree breeding. Thus, in the breeding for hardiness, early testing of the photoperiodic response of families may be used to identify clones that should be used in seed orchards aimed at production of families of outstanding hardiness.

Since *P. contorta* provenances from the same range of distribution as in the present investigation were tested in two series of field trials (Hagner & Fahlroth 1974, Lindgren et al. 1976) we found it worthwhile to study the agreement between our data and those from the two reports cited above.

In the paper by Lindgren et al. (1976) two of the trials, Nos. 18 and 19, had average percentages of dead plants amounting to 58.9 and 67.4 %. Since the percentages in these two trials are not too far from 50 % they are suitable for a comparison with our data. First a stepwise regression analysis was carried out with the percentage of dead plants in trials 18 and 19 as the dependent variable. Five variables, latitude and altitude and their squares and cross product, were used as independent variables. The calculations were limited to the 34 provenances within the latitude range of 47–63°, i.e. the same range as the material of the present investigation. The regressions obtained are shown in Table 4.

Latitude² and altitude² were the only significant independent variables.

Of the 34 provenances, 14 originated from the altitudinal range of 700–900 metres, and 13 of these 14 from latitudes 54–63°. The rest of the provenances occurred in a scattered way distributed over several latitudes at different altitudes (cf. Table 3 in Lindgren et al. 1976).

The most reliable comparison of our data and the regressions of these two field trials would therefore be within the latitudinal range of 54–63°.

Data on survival from two of the provenance trials belonging to the Swedish Cellulose Company were kindly placed at our disposal by Dr. Stig Hagner. These two trials, No. 30, Lapträskberget (lat. 65°55', alt. 225 m) and No. 31, Volgsele (lat. 64°46', alt. 435 m) had percentages of survival that made them suitable for a comparison with our data from the climate chambers.

A stepwise regression analysis of the same type as described above was carried out to test the latitudinal and altitudinal influence

on survival. The regressions obtained are shown in Table 4.

The regression of trial No. 30 is characterized by a steeper declination in the latitudinal range of 51—55° than the other three regressions based on field data.

In Figure 28 the regressions for trials Nos. 18—19 and 30—31 are illustrated for a constant altitude of 800 m. The regression for plants in classes 3—5 treated during the second growth period at a night length of 13 h is also shown in this figure. The last-mentioned regression was the one based on our data that showed the best visual agree-

ment with the data from the field trials. The slope of the regression based on data from trial No. 31 shows a conspicuously good parallelism with the slope of the regression based on our data from the climate chamber study.

A comparison of the slopes of the regressions is the most vital one, whereas the level of the regressions of the field trials are dependent on the severity of the test locality. As may be seen from Figure 28, there is a fairly good resemblance between all the regressions illustrated in this figure.

Even if the visual agreement is good

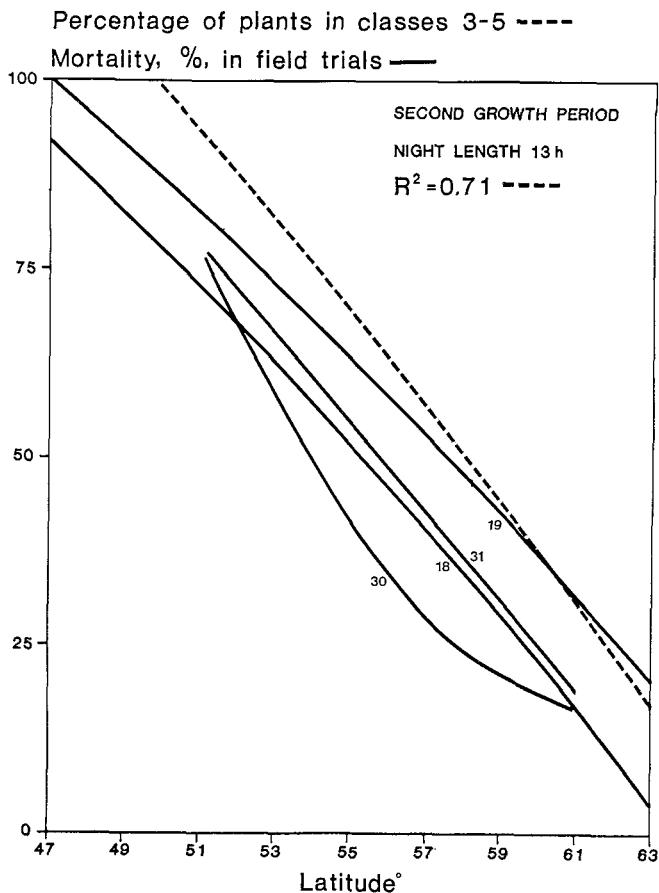


Figure 28. Regression of percentages of plants in classes 3—5 on latitude. Freeze testing at -10°C was performed after treatment with 13 h night in second growth period. This regression obtained in a phytotron is compared to regressions of percentage mortality on latitude for field trials 18 and 19 (based on results obtained by Lindgren et al. 1976) as well as trials 30 and 31 (based on results placed at our disposal by Dr. S. Hagner, Swedish Cellulose Company). The curves are drawn for an altitude of 800 m. Trials: No. 18—Kompo, Nattavaara, lat. $66^{\circ}44'\text{N}$, alt. 375 m; No. 19—Lappeasuando, Svappavaara, lat. $67^{\circ}30'\text{N}$, alt. 390 m; No. 30—Lapträskberget, lat. $65^{\circ}55'\text{N}$, alt. 225 m; No. 31—Voigsele, lat. $64^{\circ}46'\text{N}$, alt. 435 m.

between the regressions based on our data from the climate chamber study and the regressions based on field data, the goodness of agreement remains to be tested.

As may be seen from Table 4 the independent variables proving significant were not always the same. This complicates a test of the agreement between different regressions. However, in most cases, the square of the latitude was shown to be significant. The only exception to that was trial No. 31. A detailed analysis of the stepwise regression analysis for trial No. 31 showed that the latitude and the square of the latitude did not differ significantly, the latitude explaining the variation in mortality only slightly better than the square of the latitude.

Since the square of the latitude in all cases played a dominating role, the comparison of the regressions based on our data and the data from the field trials could best be carried out by a statistical evaluation of the agreement between the coefficients for the square of the latitude.

In the regressions in which other independent variables are proving to be significant, they will influence the numerical value of the coefficients for the square of the latitude. To circumvent this problem

a separate calculation of regressions was carried out with the square of the latitude as the only independent variable. The regression coefficients thus obtained were analysed by conventional t-testing. Before this t-test was carried out, an F-test of the difference of the residual variances of each of the regressions compared pairwise was performed (cf. Table 8). For those cases in which significant differences were obtained as regards the residual variances, the possibilities of revealing significant differences between the two regression coefficients are reduced. When analysing the results in Table 8 this must be remembered.

When no significance is obtained in such a type of t-test one can only conclude that there is no statistical proof of differences between the regression coefficients compared; one cannot conclude that they are alike.

When analysing the data in Table 8 it is evident that the data from the climate chamber study in many cases are not significantly different from the data of the field trials. The best agreement was obtained after treatment of the plants during the first growth period at 13 h and estimating the injuries by the percentage of plants in classes 4—5, as well as testing at

Table 8. Test of agreement of the square of the latitude of the regression based on climate chamber data on the one hand and the square of the latitude based on four field trials on the other hand.

Field trials	Percentage of plants in classes 4—5						Percentage of plants in classes 3—5					
	1st growth period			2nd growth period			1st growth period			2nd growth period		
	12 hours (b = -0.074)	13 hours (b = -0.045)	13 hours (b = -0.074)	12 hours (b = -0.051)	13 hours (b = -0.062)	13 hours (b = -0.057)	12 hours (b = -0.051)	13 hours (b = -0.062)	13 hours (b = -0.057)	12 hours (b = -0.051)	13 hours (b = -0.062)	13 hours (b = -0.057)
residual variance	lat. ² residual variance	residual variance	lat. ² residual variance	residual variance	lat. ² residual variance	residual variance	lat. ² residual variance	residual variance	lat. ² residual variance	residual variance	lat. ² residual variance	
18 (b = -0.043)	0	0	0	0	0	*	0	0	0	0	0	0
19 (b = -0.040)	0	*	0	0	0	*	0	0	0	0	0	0
30 (b = -0.046)	**	(0)	0	0	**	(0)	0	0	*	(0)	*	(0)
31 (b = -0.049)	*	(0)	0	0	*	(0)	0	0	0	0	0	0

12 h night but estimating the injuries by the percentage of plants in classes 3—5. The coefficients of latitude² for these two regressions were numerically closest to the ones from the field trials, as may be seen in Table 8.

The fact that the regressions obtained for our material mostly had steeper slopes than the ones for the field trials means that a higher resolution between different genetic entries can more easily be achieved in climate chambers than in field trials. However, if the slopes of field and climate chamber material differ too much, a direct allocation of material to different breeding zones is hampered.

Based on our results, how should early testing be designed in order to serve forest tree breeding? In the early tests a series of standard populations, whose performances are known from field trials, must be included. Moreover, the standards must represent a wide range of hardiness. The material should be cultivated under the same regime as in experiments IV and V and freeze tested at a night length of 12 or 13 hours. A quotation from the paper of McCreary et al. (1978) is valid for our recommendation as well. It is as follows:

“Similar photoperiodic treatments but different temperature, humidity or soil moisture conditions might cause seedlings to respond differently. For this reason, our recommendations apply only to greenhouse environments similar to those used in these experiments.”

A slightly less accurate estimate of the frost hardiness is obtained if dry matter content, lengthening of secondary needles or anthocyanin colour is assessed without an accompanying freeze testing. According to the performances of the previously untested genetic entries in relation to the standards they may be allocated to the different breeding zones.

Similar suggestions to screen for hardiness in *Pinus sylvestris* were published ten years ago by Hagner (1970a).

Summary. In conclusion it may be stated that there are good possibilities of using climate chamber studies for identifying genetic entries of desired hardiness under field conditions. An identification of material adapted to the conditions in different breeding zones can also be carried out by the aid of early testing of the type used in the present investigation.

Acknowledgements

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Sammanfattning

Syftet med föreliggande rapport kan sammanfattas på följande vis:

1. Att bestämma foto- och termoperiodiska krav hos olika populationer för uppnående av frosthärdighet.
2. Att undersöka ett antal karaktärer för att se om någon eller några av dem är lämpliga som indikatorer på frosthärdighet.

Det geografiska ursprunget för de 15 populationerna som ingår i undersökningen framgår av Figur 1 och Tabell 1. Odlingsbetingelser och tidpunkter för frystestning för de olika experimenten framgår av Tabell 2.

Resultat

Härdighet och yttre förhållanden. Ju längre nattlängd desto bättre härdighet uppnåddes hos plantorna. Detta skedde oberoende av om plantorna odlades under en successiv nattförlängning eller om de odlades parallellt under olika fotoperioder. Fotoperioden är uppenbarligen den faktor som sätter igång utvecklingen av härdigheten. Andra faktorer såsom nattetemperaturen påverkar också utvecklingen av härdigheten. Det är de låga nattetemperaturerna ($< +10^{\circ}\text{C}$) som stimulerar denna utveckling.

Genetisk variation i härdighet. En stor genetisk variation i frosthärdighet kunde konstateras i vår undersökning. Ett starkt inflytande av latituden på härdigheten dokumenterades klart i de regressionsanalyser som utfördes. Ju nordligare ursprung desto

bättre härdighet erhöles. Höjdläget föreföll också att ha en viss betydelse såtillvida att de högst belägna populationerna hade en bättre härdighet än populationer från lägre höjdlägen vid en och samma breddgrad.

Resultaten visade att man kan få en god separation av olika populationer med avseende på härdighet om man använder de av oss utnyttjade odlingsbetingelserna med successiv nattförlängning och genomför frystestningen vid en nattlängd av 12 eller 13 timmar.

Karaktärer som är korrelerade med härdigheten. Starka samband erhöles mellan frostskador och följande tre karaktärer:

- sträckningstillväxten hos dubbelbarren
- torrsubstanshalten hos terminal- eller sidokottens översta delar
- antocyanfärgen.

Samband mellan våra data och data från fältförsök. För denna jämförelse utnyttjades resultat från två av skogshögskolans försök samt resultat från två försök tillhöriga SCA, som ställts till vårt förfogande av docent Stig Hagner, vilket vi med stor tacksamhet erkänner. Regressionerna från fältförsöken och vår egen undersökning i fytotronen visade god överensstämmelse med varandra (jfr Figur 28). Odling och frystestning av familjer och populationer hos *P. contorta* enligt vad som skett i vår undersökning kan med fördel användas för att rangordna dem med avseende på härdigheten. Genom att inlemma material med känd härdighet i en dylik testning bör det vara möjligt att fördela material med ökad härdighet till skilda förädlingszoner.

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