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THE COLD HARDENING OF ONE-SEASON DOUGLAS-FIR AND LODGEPOLE PINE SEEDLINGS UNDER ARTIFICIAL CONDITIONS

by
Roger Timmis

PACIFIC FOREST RESEARCH CENTRE
CANADIAN FORESTRY SERVICE
VICTORIA, BRITISH COLUMBIA

INTERNAL REPORT BC-35

DEPARTMENT OF THE ENVIRONMENT
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PREFACE

This report summarizes the results of research investigations on low temperature conditioning of young coniferous seedlings performed by Mr. R. Timmis during three summers' employment as a Graduate Assistant at the Pacific Forest Research Centre (1968-1970). His initial assignment was to review the literature on cold hardiness and to experiment sufficiently with seedlings in their first growing season to confirm whether or not known principles of environmental conditioning apply to container-grown nursery stock. The initial goals were soon reached and, as the contents reveal, investigations were extended to delve into some of the physiological fundamentals of low temperature conditioning. This, and additional work, was undertaken by Mr. Timmis as part of his graduate program at the University of British Columbia. Some of the results reported here form part of his Doctoral dissertation currently being submitted to the Faculty of Forestry, but much of the material may not be reported elsewhere. Therefore, it is collected for the benefit of future workers.

Mr. Timmis prepared much of this report over a year ago, but it has not been released lest it prejudice his graduate thesis. The list of references has not been brought up-to-date, although several pertinent reports have been published since this draft was prepared. These will undoubtedly be reviewed by Mr. Timmis in several papers arising out of his thesis which he presently plans to submit for publication. Mr. J. T. Arnott of the Pacific Forest Research Centre is credited with guiding the report through to completion as a goal of Study PC 088: culture techniques for the production of container seedlings.

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THE COLD HARDENING OF ONE-SEASON DOUGLAS-FIR AND LODGEPOLE PINE SEEDLINGS UNDER ARTIFICIAL CONDITIONS

by

Roger Timmis[1]

1. INTRODUCTION

This report describes experiments carried out in 1968-69 with the aim of determining, empirically, conditions that would favor the development of frost hardiness in bullet-grown seedlings, and thus ensure better survival after outplanting in the fall.

A survey of literature at the beginning of the study showed that although the environmental (and some physiological) factors affecting cold hardiness had been determined, much of the work was done on herbaceous plants or woody plants of horticultural interest. The responses of conifers were incompletely known, particularly in the age and size range preferred for container planting. Studies on 2-year-old seedlings then in progress by the B.C. Forest Service (and subsequently published by Van den Driessche, 1969) promised some information relevant to the acclimatization 'recipes' sought, but did not examine the available range of conditions, nor relate results to possible timing of treatments during seedling maturation.

A certain amount of literature published since the initial submission of this report (December, 1970) has not been reviewed in discussing the results, but it does in general support them.

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Experiments were carried out during or after the first (sometimes the second) season's growth, mainly on a coastal and an interior provenance of Douglas-fir (Pseudotsugae menziesii (Mirb.) Franco), and on interior lodgepole pine (Pinus contorta Dougl.), all of which had been directly sown into the containers. The more recent experiments concentrated upon the coastal Douglas-fir which, while reflecting the trend of other plants, proved more sensitive to treatments and were relatively uniform in responses to freezing.

Four series of experiments examined the effects of six factors (temperature, daylength, light intensity, moisture, nutrition and duration of treatment) in various factorial combinations, with some correlation being made to stage of growth.

2. DESCRIPTION OF EXPERIMENTS

Experiment 1 The first experimental series was carried out from July to November, 1968, in three cold rooms maintained within a ±1.5 C temperature range, at averages of 1, 5 and 10 C. In each cold room, a plywood growth cabinet provided 100 ft-c of incandescent light in each of three chambers; one for 8 hours, one for 12 hours and one for 16 hours a day. A forced draft of cold air ensured that day temperatures under the low light intensity in the chambers exceeded the cold room temperature by only 1.0-1.5 C. This arrangement provided nine climatic conditioning treatments under which plants were placed at 3 times during the later part of the growing season. A control was provided by plants retained in a shadehouse (80% light) under natural temperature and daylength conditions. Plants retained in a growth room during the earliest treatment furnished an additional 'control' for that particular case.

All seed was sown in a peat-sand mixture, covered with granitic gravel in rigid 4½ x 3/4 inch bullet-shaped plastic containers. The seedlings were raised in the shadehouse, watered daily and supplied with NPK fertilizer regularly by subirrigation. Those retained as controls continued to receive this treatment, which was needed to maintain health under outdoor conditions, while those placed in the humid cold rooms received no further feeding and required watering only once a week. In both cases, seedlings appeared green and healthy before the freezing tests. The possible effects of this difference in feeding treatments were subsequently examined in Expt. 4.

Altogether in this experiment 2 species, 3 provenances and 2 ages of stock were compared. These were:

1.1 Douglas-fir, Prince George provenance (B.C. Forest Service seed lot #590) sown March 1968

1.2 Douglas-fir, Prince George provenance (#590) sown May 1968

1.3 Douglas-fir, East Vancouver Island provenance (#315) sown May 1968

1.4 Douglas-fir, West Vancouver Island provenance (#516) sown 1967

2.1 Lodgepole pine, Prince George provenance (#55) sown March 1968

2.2 Lodgepole pine, Prince George provenance (#55) sown May 1968

Only young coastal Douglas-fir (1.3) was employed as the basic subject throughout all factorial combinations. Young interior Douglas-fir and pine were used in all except the September treatment. The use of the few older seedlings was further restricted. This allocation of plants to treatments is shown in Figure 1. A $6\frac{1}{2}$-week treatment was also tested in this experiment, although the range of shorter durations was not examined until Expt. 4.

Most experimental units contained six one-plant replicates selected for uniformity from the available stock. At the beginning of the July treatment, all May-sown plants were very small (4-5 cm), possessing no terminal buds and only occasional minute laterals. The pine bore only primary needles in this and all subsequent experiments. The plants used for the August treatment were stockier, 1-2 cm taller, with more distinct lateral buds and only occasional minute terminals. By mid-September, the setting of terminal buds on plants before treatment was quite obvious; the young plants had taken on a woodier appearance and were 7 to 8 cm tall.

After 8 weeks, the plants were measured for bud development and height growth, and cold hardiness was evaluated after standard freezing treatments. Before freezing, the soil was saturated with water by sub-irrigation and the plants were allowed to equilibrate for several hours to bring them to a comparable state of hydration. The freezing was done in an insulated box placed in a -25 C cold room and was monitored by using two thermistor probes and 'Rustrak' chart recorders. After several hours at 0 C, during which soil water solidified, the air temperature dropped at the rate of 4 C per hour to the desired low, whereupon the box was transferred to a warm room and thawing was allowed to proceed at a similar rate. Temperature differences within the box did not exceed ± 1 C because air was circulated by a small fan. The low temperature was maintained for 15 minutes. Following the thaw, plants were placed in a growth room and scored for proportion of dead or discolored leaf tissue after a 4-day recovery period. The plants were then held in the growth room for 3 months to relate damage scores to ultimate survival and growth. The validity of the cold hardiness criteria used in this and later experiments is discussed in Section 3.1.

Experiment 2 This was a small experiment carried out at the same time with some of the plant material employed in Expt. 1, to determine what difference 'normal' light intensities and extremes of moisture supply would make to the induced hardiness. Plants were placed in a cool (5 C) growth room with mixed incandescent and fluorescent light at 550 ft-c under an 8-hour daylength. Two treatments were shaded to a light intensity of 90 ft-c to resemble the cold room conditions of Expt. 1. One of the shaded treatments received daily watering, while the other was watered only lightly whenever its weight had descended to a value known from preliminary work to just precede wilting. The experiment began in early August, ran for 8 weeks, and was assessed for hardiness in the manner already described.

Experiment 3 This was begun in April 1969, with plants that had been sown in February in a growth room at the University of British Columbia in the same soil mix and container, and fed periodically with NPK. The aim was to examine a complete range of bright daylengths for a 'hardening' optimum; to establish whether light intensities higher than the 300 ft-c (reported as saturating by Zehnder and Lanphear, 1966) would be worthwhile and to provide a range of hardiness levels for some biochemical and anatomical tests. The electrical conductivity of leaf ex-diffusate was used as a more objective measure of frost injury, expressed as in Dexter's original method (1932) as a percentage of conductivity after boiling and under carefully standardized conditions.

The experiment took place in a cold room maintained at 1 ± 1.5 C under daylengths of 0, 1, 2, 4, 8, 12, 16, 20 and 24 hours. 800 ft-c was provided to each light-tight compartment by 2 fluorescent tubes (one 'cool white' and one 'Gro-lux') 20 cms above the plant tops. External fans maintained air around the plants at 1 C above ambient during the day. Single and double layers of shading cloth reduced light intensities to 200 and 50 ft-c, respectively, in subsections of the 8-hour and 16-hour compartments. Under each treatment, 12 coastal Douglas-fir were placed for 6 weeks, with equal numbers of interior Douglas-fir and pine being inserted under selected treatments where space was available.

The assessment employed a number of frosts to ensure that a range of injury was obtained. The procedure was to remove two 'middle-aged' needles from each plant and store them under formalin-acetic acid for later sectioning, then to subject the seedlings to freezing at -4, -8, -12 or -22 C (4 plants in each of 3 frosts). After thawing, a 'vertical' sample of 7 needles was removed from one side of each seedling and placed in 5 ml distilled water in a vial. The vials were gently shaken for a 12-hour period in a water bath at 30 C, after which conductivity of the solution was recorded. Water bath temperature was then raised to 95 C for 5 minutes to kill the tissue. (Water loss from the vials was prevented by a press that held caps in place). After a further 6-hour period at 30 C, final conductivity was determined as a base for percentage determinations. Half of the seedlings, i.e., 2 from each of the 3 frosts for each treatment and all minus 9 needles, were placed in a growth room for damage score assessment and final survivals. The other half were cut up and freeze dried for later protein and amino acid analysis. The latter group also furnished root, stem and leaf dry weight measurements as a measure of growth, if any, during the treatments, and an 1/8-inch stem segment for later sectioning and lignification tests.

The control consisted of plants retained in the growth room which, unlike the cold room stock, continued to receive some NPK. At the beginning of the treatment, terminal buds had been set on the pine but not on the Douglas-fir, though small laterals were common, and the seedlings had reached a stage of development similar to those of Expt. 1 in early August.

Experiment 4 The fourth investigation was conducted on the coastal Douglas-fir used in preceding experiments and also on a high elevation south coastal provenance (B. C. Forest Service seedlot no. 327), both sown in mid-April 1969 and raised under the standard conditions. The aim was to test shorter durations of treatments and the effect of fertilizer application both in the cold rooms and outdoors. Plants were placed under a 300 ft-c, 10-hour day, incandescent light in the 1 C cold room at weekly intervals beginning August 7, and were scored for hardiness following frosts applied on September 4 and 13. Fertilizer treatments consisted of weekly subirrigation by 2-3 oz of fertilizer per 100 gal water (4-6 ppm) according to the regime shown in Table 1.

RESULTS AND DISCUSSION

3.1 Validity of hardiness criteria

The proportion of damaged needle tissue as a method of frost damage to whole plants was adopted in these experiments because it was quick, demonstrative, consistent (as used by one investigator) and

Table 1. Fertilizer treatments used in Experiment 4

Treatment	Week 0 (Aug. 7)	Week 1	Week 2	Week 3	Week 4	Week 5
shadehouse normal care	a mixed complete nutrient fertilizer 'Plant Prod', supplied by Plant Products Ltd. Ontario (15-15-30)			Ammonium sulphate (21-0-0)	Amm. sulph. (21-0-0)	water only
shadehouse decreasing N increasing K	Plant Prod (15-15-30)	Muriate of Potash (KCl) (0-0-60)	M.P. (0-0-60)	M.P. (0-0-60)	Amm. sulph. (21-0-0)	water only
cold room 5 weeks	Plant Prod (15-15-30)	Ammonium nitrate (34-0-0)	Amm. nitr. (34-0-0)	Amm. sulph. (21-0-0)	Amm. sulph. (21-0-0)	water only
cold room 4 weeks	Plant Prod (15-15-30)	Amm. nitr. (34-0-0)	Amm.nitr. (34-0-0)	Amm. sulph. (21-0-0)	Amm. sulph. (21-0-0)	
cold room 3 weeks	-	Amm. nitr. (34-0-0)	Amm.nitr. (34-0-0)	Amm. sulph. (21-0-0)	Amm. sulph. (21-0-0)	-
cold room 2 weeks	-	-	Amm.nitr. (34-0-0)	Amm. sulph. (21-0-0)	Amm. sulph. (21-0-0)	-
cold room no nutrient	water only	water only	water only	water only	water only	water only
				Planting out at Mead Ck, and Copper Canyon, ten-plant samples photographed and dry-weighed.	frost testing of 4-week sample	frost testing of 5-week sample

provided a range of values. Subjective error in classifying seedlings was tested by two repetitions of one of the assessments, run consecutively, after re-randomization. In all cases, damage scores were within one unit of each other, the total range being from 0 to 10 units, and conclusions concerning the treatments remained unchanged. A typical range of values provided by this assessment is illustrated in Fig. 9, Section 3.3

Not much use is made of scoring methods in the literature because larger and broadleaved branched plant material is more difficult to deal with in this way and because the method is subjective. Both Day and Barrett (1962) and Glerum _et al._ (1966) have found it satisfactory for conifers. The more usual procedure for crop plants of various sorts is to use the percentage of plants surviving a frost as a measure of a hardening treatment's effectiveness, but this requires much replication. Another method employs an indirect criterion of injury such as the elution of amino acids or mineral ions from the tissue by distilled water. Using the last method with Douglas-fir, Van den Driessche (1968) showed that a good prediction of final survival by stem ex-diffusate could be obtained. Zehnder and Lanphear (1966) also obtained a close correlation of percent diffusate with observed injury in _Taxus_ needles. Figure 2 illustrates the relationships obtained in the present investigations between the percentage ex-diffusate of the leaf tissue sample, the damage score and the final survival, for all the coastal Douglas-fir measured in Expt. 3. Means of groups defined by 5% intervals across the range of conductivity values, or containing a constant number of observations across this range (Fig. 2b) show a high correlation between the two measures used. Interior Douglas-fir (Fig. 3) exhibits a similar relationship when individual values are plotted for the 22 C frost (the only one to produce a range of damage in this case). However, the point at which half the coastal plants ultimately survive occurs in the lower fifth of either damage score or conductivity range (2 units and 11%, respectively). This means that even with most of the foliage undamaged, the plant is still likely to die after freezing under the described conditions. Excavation showed that this was because the root system had been killed (even when a vermiculite covering of the containers was tried) and, as Olien (1967) points out, this does not produce visible effects on the foliage for several weeks.

Numerous early reports (cited by Parker, 1963) and more recent ones (Zehnder and Lanphear, 1966; Pellet, 1966) have shown that the roots are relatively frost tender even in mid-winter. Therefore, when overwintering of plants in containers exposed to the air is being attempted, needle damage is of no use as a predictor of survival. In fact, all the literature on root hardiness quotes -12 to -15 C as the absolute lower limit tolerated. In view of this, the observed survival of containerized Douglas-fir and lodgepole pine seedlings above ground, with only a light snow cover, at air temperatures of -40 C in Prince George, indicates a need for research on root hardiness _in situ_.

An additional cause of poor correlation with survival is the deficiency of mature lateral buds to initiate shoot growth from the lower undamaged portions of the stem. Nevertheless, in the absence of a simple measure of bud hardiness, leaf damage is considered an adequate criterion because, under natural fall conditions after planting, the roots will escape freezing during frosts and buds will be better developed. Under these conditions, needle damage provides the first indication of frost susceptibility and of economic if not biological survival.

A shortcoming of all hardiness measurements based on a single frost is that these may fail to differentiate between plants that are all highly tolerant or intolerant of that degree of frost, while actually differing in the temperature at which damage is first inflicted. This is exemplified by the two levels of frost used to test the 'early-August' treatments of Expt. 1 (Fig. 4). From the -8.5 C frost, we would conclude that the two lower temperature conditioning treatments did not differ in their effectiveness, irrespective of photoperiod, whereas the -16 C frost shows that both photoperiod and temperature factors had a significant effect. Although several freezing temperatures were used in the present experiments, these have not always been at the most sensitive level for discrimination and therefore treatment effects were not as definite as they might have been (the accidental lack of a -16 C frost in Expt. 3 places it in this category).

One reason for the relatively small number of frost temperatures employed is that the number of replicates and the time needed increases proportionately with the number of frosts. If precise predictions of seedling survival in relation to expected climatic conditions at planting sites are needed to 'optimize' cultural and planting schedules, it is necessary to test a complete range of frost temperatures to ascertain, for each treatment, the one which guarantees the desired probability of survival. One can get this type of data most easily by using detached needle samples, provided that a reliable correlation with 'whole plant' survival can first be obtained. Steponkus and Lanphear (1967) used such a procedure, measuring damage by tetrazolium staining and determining a "killing temperature" based on 50% organ survival, but without reference to whole plants. This "killing point" procedure is feasible in the context of the current container planting development, especially with the design of a temperature gradient freezer which would allow the range of frosts to be applied in a single test. But for the objectives of the investigations reported here, the purely comparative and somewhat subjective criterion described in section 1 is valid.

3.2 Sources of variation - a summary

The variables examined can be considered as environmental, developmental or genetic (species - provenance), though this distinction cannot persist on a fundamental level. Each category accounted for a large amount of variation in hardiness of the experimental plants. Each is therefore important in defining a set of hardiness-inducing conditions suitable as a cultural practice.

The scope of this variation is illustrated in Figs. 4 and 5. Differences between blocks of solid histograms are developmental (incorporating 4 levels of test frosts); differences between the 10 columns of a block are environmental (illustrating in this case the two most important climatic variables, temperature and photoperiod); differences shown by shading within the columns are genetic at the species and provenance level, and differences within a column subdivision (illustrated by Fig. 5) are mainly genetic at the individual level. In the analyses of variance performed on these data, the last-mentioned "between tree" variation was used as the error term. Following treatment, the coastal Douglas-fir ranged in hardiness from those 100% damaged by a -8 C frost to those sustaining only 10% injury after a frost at -25 C.

Temperature was the most important of the environmental variables. All tested species, provenances and ages acclimatized better at lower temperatures. In Figs. 4 and 5, the coastal Douglas-fir in each block exhibits a range from more or less undamaged by the test frost to severely damaged or completely killed, corresponding with the 1 and 11 C conditioning treatments. The same cannot be said of the extremes in photoperiod, nor of light intensity in later experiments. Photoperiod and light intensity are therefore of secondary importance, although some light is needed to allow any hardening to take place at all. These last two factors interacted, presumably due to the independent involvement of both hormonal and photosynthetic mechanisms. The effect of cool temperature, on the other hand, appeared to be more closely linked to the seedlings' stage of development and is discussed below in connection with this. Nutrient and moisture supply were of least importance, though both had a measurable and positive effect on cold hardiness under some conditions.

The timing of treatments from sowing date; that is, in relation to developmental stage, also imposed a consistent pattern upon purely environmental responses. This is seen by comparing 'July' with 'August' blocks at -8.5 C in Fig. 4, and 'August' with 'September' blocks at -16.5 C. Again generalizing in the face of some specific exceptions, it can be seen that treatments were more effective if begun later. The controls illustrate the natural progression of hardiness during this interval and suggest, in spite of an anomalous but statistically non-significant reverse trend in the coastal Douglas-fir, that the conditioning treatments have an additive effect upon the hardiness condition obtained naturally. This discussion is expanded in Section 3.3 in relation to the parameters of seedling maturity measured among the environmental treatments. An age effect of foliage in a corresponding state of development but on 2-year, instead of 1-year-old plants was observed, but could not be substantiated statistically on the small number of variable plants used. The duration of a treatment was found to be important up to a period of 5 weeks, beyond which time little further benefit was shown at the mild -8C frost temperatures used. The benefits of continued feeding in the cold room did not become apparent until the 4-week period was exceeded.

Genetic differences between the species pine and Douglas-fir are also exemplified by the sample of data illustrated in Figs. 4 and 5. The fir is hardier than pine from the same seed area, with few exceptions. The interior Douglas-fir provenance is hardier than the coastal and also hardens faster when left outdoors. A Douglas-fir provenance from the west coast of Vancouver Island retained complete susceptibility to frost (100% damage) during the July conditioning treatments of Expt. 1.

The following sections present data in support of these statements and consider the results more fully with regard to literature, interpretation and practical application. Differences and correlations discussed are statistically significant unless otherwise stated, though the numerous analyses of variance and response surface are not presented.

3.3 Temperature, timing and growth relationships

The seedlings placed under conditioning treatments in mid-July were small, rapidly growing and without buds. Those kept at 6 or 11 C underwent appreciable growth and lateral bud development during the 2-month treatment. Photoperiod had no effect on this development (Table 2), except in the warmest temperature where terminal buds developed under the two longer day lengths. However, the lesser development under low intensity 8-hour days is considered a photosynthetic rather than a photoperiodic effect (Section 3.4). Figure 6 shows that the extent of bud development and height growth are inversely correlated with hardiness in both Douglas-fir provenances and to a

Table 2. 'Maturation', daylength and temperature relationships among seedlings of Expt. 1.

Species/	Temperature	1 C				6 C				11 C				Control
Provenance	Photoperiod	8	12	16	mean	8	12	16	mean	8	12	16	mean	mean
Coastal	lateral buds/plant	1.0	0.2	0.5	0.57	2.5	1.8	1.0	1.77	2.7	2.7	4.7	3.37	8.7
D.-fir	Avg terminal bud size	0	0	0	0	0	0	0	0	0	0.7	1.0	0.58	3.0
(1.3)	Avg height (cm)	4.7	4.3	5.2	4.7	5.5	5.3	5.3	5.4	7.5	7.0	7.5	7.3	6.8
Interior	lateral buds/plant	2.5	3.8	3.2	4.75	5.5	4.7	5.8	5.33	4.5	5.7	7.8	6.00	7.2
D.-fir	Avg terminal bud size	0	0	0	0	0	0	0	0	0	3.0	5.2	2.73	3.8
(1.2)	Avg height (cm)	5.2	5.0	5.3	5.2	5.7	5.5	6.0	5.7	6.0	6.8	7.0	6.6	7.3
Interior	lateral bud-plant	0.8	0.7	0.5	0.63	0.8	1.5	1.0	1.10	1.3	1.5	1.5	1.43	3.3
Lodge.pine	Avg terminal bud size	0	0	0	0	0	0	0	0	0	0	0.8	0.27	3.3
(2.2)	Avg height (cm)	5.5	5.7	5.8	5.7	6.7	6.0	6.5	6.4	6.8	5.8	6.3	6.3	6.7

Notes: 1. Values given are the means of six plants. 'Means' given are thus derived from 18 plants.

2. Terminal bud size classified subjectively between 0 and 5.

lesser extent in the pine. Thus hardiness was gained at the expense of completing the season's normal development. A further observation was that red anthocyanin pigmentation of the stems was greater in the hardier Douglas-fir seedlings. Stem color ranged from deep mauve through pinkish-green to more or less green (in controls), and might offer a direct indicator of hardiness by extraction and colorimetric measurement. This would be in line with Parker's correlation in English ivy (1962) and Van Huystee's observations of red osier dogwood (Van Huystee _et al._, 1967).

The morphological development of the September-treated plants was similar to that of the controls in Table 2; that is, small terminal buds were set, the stems were stockier and the needles more rigid and open in habit. It is not known if these seedlings had entered winter dormancy because no specific growth test was made at this stage, but it may be presumed that they had at least begun to do so for two reasons: (1) Lavender's criterion of moderate terminal bud development in Douglas-fir was met (Lavender _et al._, 1968), and the time elapsing before initiation of dormancy in his plants was well exceeded by the seedlings in the present investigation, and (2) similar plants transferred to a growth room at about the same time for other purposes, flushed erratically only after several months. The same cannot be said of the July-treated plants for reasons converse to (1) and (2) above, and because uninjured experimental plants, during the post-freezing recovery period, set buds and flushed with much less delay. Both dormant and non-dormant groups attained considerable hardiness, and exhibited, as shown in Fig. 4, a similar response pattern to climatic treatments (the August treatments occupied an intermediate position). Therefore, these results confirm for conifers those obtained by Irving and Lanphear (1967) for _Viburnum_, in showing that the development of hardiness proceeds independently of bud setting or dormancy. The failure of the July- and August- treated seedlings to mature shows that the bud set and dormancy processes are suspended or retarded by the low temperatures most favorable to hardening.

The literature on cold acclimation and stage of growth, relevant to the timing of conditioning treatments, offers no unambiguous and consistent picture of these relationships. Contradictory results seem to coincide with the division of experimental material into broad-leaves and conifers, indicating that the sequence of events may be different in the two types, even though basically the same kind of biochemical changes have been finally brought about (Siminovitch *et al.*, 1967 versus Pomeroy *et al.*, 1970). There is strong evidence in the broadleaved woody plants that hardening comes about in three stages: (1) entry into dormancy induced by short days; (2) development of hardiness at temperatures around 0 C in light, and (3) a deepening of hardiness at sub-zero temperatures, which is able to proceed in the dark. Tumanov *et al.* (1963) found that both birch and acacia could be obtained in any one of these stages after the appropriate conditions had been applied. The 50% killing temperatures for the birch were: -5 C (growing), -15 (dormant only), -20 (stage 2 only), -5 (stage 3 only) and -60 C (all stages sequentially). Van Huystee *et al.* (1967) demonstrated a similar 3-stage situation in red osier dogwood by examining both natural and artificial conditions. Earlier work by Sakai (1955) suggests that mulberry also follows this pattern. However, not all broadleaved species have produced such clear results. Ivy and *Viburnum* gain only slight hardiness (3 - 6 C) by becoming dormant (Irving and Lanphear, 1967), and the authors do not suppose that this is due to dormancy *per se*.

In conifers, the third stage has not been demonstrated by two attempts out of three, nor has the existence of the first two sequential stages been consistently shown. The responses of potted *Taxus* seedlings to short-day pre-treatment (Zehnder and Lanphear, 1966) can be interpreted in terms of a 2-stage process, but this is not confirmed by Van den Driessche's results (1969), albeit at a rather low light intensity. Van den Driessche further noted an increase in dormant apices with hardiness, but could not conclude that the dormancy itself conferred hardiness. In neither case was there a tertiary effect of sub-zero temperatures. In contrast to this are results from Germany (Scheumann and Bortitz, 1965) that provide evidence for Tumanov's 3-stage sequence of events occurring in both Norway spruce and Douglas-fir. A recent abstract by Glerum (1970) reports that the hardening of seven coniferous species outdoors during the fall "appears to consist of two stages: one of gradual hardening, followed by one of rapid hardening". In the absence of climatic data and various controls, together with morphological observations, this does not constitute evidence for separate physiological stages. The acceleration of a single process controlled only by one factor could account for this type of observation.

In the present investigation, the data give little support for a sequential 2-stage hardening process in which the second stage cannot proceed before completion of the first, because the photoperiod and temperature factors supposed to induce stages 1 and 2, respectively, and exclusively, are effective in increasing hardiness if applied together. Discounting the coastal provenance (for which differences in seasonal hardiness between controls were non-significant and confounded with poor health), the data show a natural development of frost tolerance in Douglas-fir controls between the mid-September and mid-November assessments (Fig. 7). This is of a similar general magnitude to the hardiness obtained by seedlings under the more unfavorable conditioning treatments (Fig. 4) but, unlike these, is positively correlated with an increase in seedling maturation. Nevertheless, the gain in hardiness cannot be ascribed to dormancy *per se*. No evidence for a third stage at sub-zero temperatures was sought because any adaptation to lower limits than those already obtained is unnecessary for fall planting.

The results obtained from Expt. 1 (Figs. 4, 6 and 7) can be summarized most simply in relation to seedling development by supposing a principally temperature-dependent progression through three independent phases: extension growth, bud set/dormancy, and single-stage hardening (Fig. 8). The descending stippled line within the temperature/time axis represents the drop in temperature of controls, and portrays the amount of time spent by these plants within the temperature range of each rather arbitrarily-positioned growth phase (the time axis is positioned from a May sowing date in this case). Thus, extension growth and bud maturation proceed to completion before much hardening of controls, whereas those plants treated at 1 C before August enter the hardening range without spending any time at the intermediate temperatures necessary for growth and bud set. If 1 C is applied in September, there is little loss of bud growth. Similarly, early treatments require a lower temperature to attain a given degree of hardiness; 6 C in September approximates 1 C in July. We may presume this relationship extends into November, but not beyond. A constant 11 C does not permit hardiness to develop at all within an 8-week period, so the outdoor seedling fares better in this respect; 11 C in September causes a loss of hardiness. All the significant correlations discussed earlier can be placed within this empirical model which, with further modification and statistical support, would provide a useful guide for cultural practice.

The omission of photoperiod from Fig. 8 is justified on the grounds that its importance in affecting growth and maturation was not demonstrated in the experiment (Table 2). Work by Vegis (1963) showed that daylength conditions can be regarded as only modifying what is basically a temperature response in dormancy, and Lavender *et al.* (1968) found in their comprehensive study that "*Pseudotsuga menziesii* var. *menziesii* appeared not to be strongly photoperiodic in nature". Even in relation to hardiness, both Zehnder and Lanphear (1966) and Van den Driessche (1969) report that photoperiod merely hastens the cold acclimation that would ultimately develop. Irving and Lanphear (1967) agree. Thus, while artificial short days are desirable for quicker results in practice, their exclusion from the diagram as a third axis does not devalue it. The shortening of days that occurs naturally *is* incorporated, together with any endogenous rhythm, as the slope of the growth stage boundaries in relation to the horizontal seasonal axis. This is a necessary inclusion to account for the development under constant conditions. In view of the narrow range over which factors were examined, the comparative rather than the quantitative measure of frost hardiness used, and the limited extent to which hardening was followed beyond that needed for autumn protection, it is not valid to extrapolate beyond the experimental conditions and time periods illustrated.

It is appropriate to note here that a 6-week period of conditioning allowed a similar amount of tolerance to develop to light frosts (Fig. 5), The results of Expt. 4 (Fig. 9) indicate that 80% of this resistance was acquired after only 4 weeks, and almost 100% after 5 weeks. Further hardening continued to occur after 5 weeks, however, to an extent that protected 8-week 1 C treatments (Fig. 4, block 3) from 16 degrees of frost fatal to the 5-week plants. Conditioning for a period of less than 2 weeks was without appreciable hardening effect. This agrees with Sakai's results on short-duration conditioning of Mulberry (Sakai, 1956). The noneffective period is indicated by the dotted portion of the 'treatment' lines in Fig. 8. The duration effect is summarized in Fig. 10.

The practical message from these temperature-growth relationships is obvious. Hardening treatments intended to adapt plants to planting in the fall should begin when seedlings have completed their growth and bud formation. Apart from allowing the seedling to grow to maximum size and nourish a strong terminal bud for next season's elongation, this ensures that the cold hardiness after a 5-to 7-week period of low temperature treatment will be greater.

3.4 Photoperiod and light intensity

The effect of daylength, as shown by the low light intensities of Expt. 1, was to increase hardiness only at the intermediate 12-hour level, suggesting that a photosynthetic deficiency was limiting the process at the normally favorable 8 hours. This is clarified by the results of Expt. 3. Figure 11 shows that 16-hour days are inhibitory at each of 3 light intensities, according to both conductivity and damage score criteria after both frosts. The 8-hour day is most beneficial.

The effect of light intensity is equally distinct. Plants under 50 ft-c achieve only a fraction of their full (800 ft-c) hardiness, while plants grown under 200 ft-c occupy a somewhat variable intermediate position. In general, the difference between 800 and 200 ft-c levels is much less important than the increment from 50 ft-c to 200 ft-c. This is in agreement with most literature on the subject (Steponkus and Lanphear, 1968; Zehnder and Lanphear, 1966).

A comparison of the whole range of bright photoperiods for hardiness induction is shown in Fig. 12, again using percentage conductivity as a more objective measure of injury. The freezing response surface shows a trough corresponding, at the two lower frost temperatures, to the 8-hour photoperiod, and broadening out under the less discriminating -8 C frost. This increased hardiness under 8-hour days is highly significant. Figure 13 shows confirmatory trends for the interior provenance and for lodgepole pine, although the minima are less well-defined. The long-day effect was partly overridden in this experiment by the 6-week duration of low temperature (contrast the effect of photoperiod under 1 and 11 C temperatures in Fig. 4), and two replicates for damage score determinations were insufficient. The data also show conductivity to be a less sensitive measure.

The simplest interpretation of Expts. 1 and 3 regarding the effects of light is that this factor affects hardening both through photosynthesis and daylength. This is well supported by the literature. On the one hand, experiments on etiolated varieties and dark-grown plants have shown a definite light requirement for hardening as an active, energy-requiring process. On the other hand, a series of papers by Irving, Lanphear and Steponkus (1967-69) have demonstrated in long-day ivy and Viburnum leaves the production of a cold hardiness inhibitor whose effect is replaceable by applied gibberrellin. The very-short-day reduction in frost tolerance in Figs. 12 and 13 is the result of inadequate photosynthesis, and is comparable with McGuire and Flint's findings for three conifers (1962). The long-day effect is probably hormonal. The hormonal aspects of both temperature and daylength stimuli are being investigated.

This result has a bearing on the problem of limited space available for large-scale cold room conditioning treatments, because vertical stacking of trays under a single light source will reduce light intensities, create a non-uniform environment, and only partially harden the stock. The disposition of incandescent lamps directly above each tier would, on the other hand, tend to cause overheating and thereby act against acclimation. Fluorescent lamps are more compact and would reduce the heating problem, but the 'cool white' type normally used radiate very little light in the red spectral band (600-750 nm). Since most known daylength effects are mediated through the phytochrome pigment system in response to red light, we must be cautious in using sources lacking this component to induce hardiness. Although the participation of the red - far red radiation balance in inducing hardiness has yet to be demonstrated it would be advisable to use the easily available red-emitting 'grolux' or 'plant-gro' fluorescent tubes in the situation described. Besides promising quicker responses to photoperiod, and therefore more rapid hardening, these sources provide a greater proportion of their output in a photosynthetically usable region.

3.5 Species, provenance and age

Comparisons among species in Figs. 4, 5, 13 and 14 show that Douglas-fir seedlings of the same age and seed area are hardier under most circumstances than those of the pine (contrary to Wentzel, 1965). There is a tendency illustrated by the 8-hour photoperiod in Fig. 14, and by certain comparisons between the data of Expts. 1 and 2, for the pine to manifest its inferior hardiness more obviously in those treatments where total light supply is low. In Fig. 14, for example, the normally unfavorable 16-hour day results in a hardier population at the lower light intensities (photoperiod x species interaction is highly significant). This could be the result of a greater debility of the more light-demanding pine when grown under dim conditions. The poorly illuminated plants also retained their initial healthy-looking green needle color, unlike the hardier well-lit groups which had turned purple at the same temperature. The lack of anthocyanin pigmentation may thus provide a more obvious indication of inadequate hardening in the pine.

The provenance differences were what one would expect, considering the respective climates of origin, and cannot be discussed profitably without further data on the interior type than is provided by the narrow range of treatments actually frost-damaged (Fig. 4). The quicker setting of buds observed in the interior seedlings indicates that the whole maturation and hardening sequence proceeds more rapidly than in coastal types, notwithstanding daylength, when temperatures descend from their seasonal peak. The relative inability of west coastal types to harden was shown by their complete intolerance to the -8 C frost regardless of July treatment, all individuals being scored between 9 and 10. The high elevation coastal type used in Expt. 4 was not significantly hardier after 5 weeks of cold room treatment than low elevation. However, significant differences between these two provenances were apparent under various nutrient conditions (Section 3.7).

Older stock was incorporated only into Expts. 1 and 2, the latter with poor replication. We are able to make two types of comparison (Table 3) with the interior Douglas-fir: (1) the newly flushed foliage develops less cold tolerance than older foliage on the same plant, and (2) there are differences between young foliage borne on one-year and two-year-old plants, but these reverse in direction according to the month (July or early August) of treatment. In the latter comparison, it appears that differences in seasonal development of the new shoots are obscuring any purely age-determined effects, such as were found by Larcher (1969) during the first five years of oak shoot development. Similarly, developmental stage confuses comparisons between old and young leaves, as is indicated by the contradictory literature. The situation under natural conditions by mid-winter is that the most recent needles survive more frost (Wentzel, 1965), but when frosts are applied during the growing season, as in Expt. 1, older needles fare best. The significantly greater hardiness attained by the lodgepole pine sown earlier in the season is an expected developmental effect. Berntsen (1967) quantifies this greater frost-hardiness in lodgepole pine seedlings with time after germination.

3.6 Moisture

Figure 15 shows that all Douglas-fir groups grown under a daily watering regime became slightly hardier on average than those that frequently approached wilting. These differences are only significant between the older interior seedlings and, in any case, are very small when compared with the light intensity, provenance, and especially the low temperature effects. Moreover, the older interior fir was the only group to suffer actual prolonged wilting and was therefore probably susceptible, mainly

Table 3. Frost damage sustained by seedlings and foliage of different ages

Treatment[3]	Interior Douglas-fir			Interior Lodgepole pine	
	1-year[1] (sown May/68)	2-year (sown May/67)		1-year[1] (sown May/68)	1-year[1] (sown March/68)
		1967[1]	1968[2]		
July, 8-week					
6°, 16 hr	0.1	0.0	2.0(1)	0.0	0.0
11°, 8 hr	2.2	0.7	9.0(4)	0.0	0.0
11°, 12 hr	0.5	0.3	3.2(2)	0.0	0.0
11°, 16 hr	3.5	1.6	7.0(3)	4.9	0.1
Control	2.4	0.0	-	2.4	1.2
August, 6-week					
6°, 16 hr	0.4	0.0	0.0(3)	0.2	0.0
11°, 8 hr	3.2	0.0	0.3(3)	4.0	2.1
11°, 12 hr	3.0	0.0	0.3(3)	0.2	0.0
11°, 16 hr	1.9	0.0	0.9(2)	4.9	3.2
Control	-	-	-	-	-

1/ Means are based on 6 and 4 plants for 8-week and 6-week treatments, respectively.

2/ Means are based on plants having new growth flush at time of testing (i.e., comparable to 1-year plants). This number is indicated in brackets.

3/ Data are taken from all Expt. 1 treatments that suffered some forest damage in at least one of the age/species categories.

because of the partial cell damage and weakening so induced.

Much of the earlier literature (reviewed by Levitt, 1956) reported that plants grown under drought conditions became hardier, though there are some contradictions. There is ample evidence that drought and frost resistance have some common basis, without necessarily being identical (Levitt, 1956; Siminovitch and Briggs, 1953). However, Van den Driessche found that the only effect of drought as a conditioning treatment on Douglas-fir was to lessen the effect of photoperiod. The conclusion, if any, to be drawn from Expt. 2 is that plants should be watered regularly during hardening to ensure that the effect of this process is not nullified by poor physiological condition. This conclusion was expressed for white pine and spruce seedlings by Fraser and Farrar (1957), "From a practical viewpoint, it would seem advisable to provide an adequate supply of soil water to conifers before the onset of freezing weather".

3.7 Nutrition

The application of fertilizer produced significant differences in hardiness which varied in direction according to provenance, treatment duration and temperature in an unaccountable way (Table 4). Part of the reason for this puzzling variation could have been prior damage due to seepage of cold air from adjacent freezing rooms left open for unduly long periods, though this mishap could not be invoked to explain shadehouse data.

The medium elevation coastal Douglas-fir (seed lot no. 315) exhibited no hardiness differences with regard to nutrient application after 4 weeks in the cold room, but after a further week there was a pronounced increase in the hardiness of the fertilized plants (from 3%

Table 4. Frost damage to two coastal Douglas-fir provenances after high and low nitrogen applications.

Treatment Combination		Medium Elevation (# 315)		High Elevation (# 327)	
		Cold Room	Shade-house	Cold Room	Shade-house
4 Weeks	High N	18.7_{abd}	94.9_{c}	61.0_{f}	40.7_{e}
	Low N	16.0_{ab}	91.8_{c}	4.7_{a}	68.9_{f}
5 Weeks	High N	3.3_{a}	99.0_{c}	16.2_{ab}	100.0_{c}
	Low N	33.9_{bdc}	98.5_{c}	27.8_{bde}	39.1_{de}

1. Each figure represents total damage to ten seedlings.
2. Shadehouse 'low nitrogen' treatments were also accompanied by higher potassium applications.
3. Any two total differ significantly at the 5% level if they do not have a common letter in their subscript.

damage to 34% damage by the 8 degrees of frost). The effect of no feeding seems to have been one of slowing down the hardening process, since the seedlings of other experiments gained considerably greater hardiness in 8 weeks in spite of being unfed. Differences in the N/K balance supplied to seedlings outdoors during this period were not accompanied by measurable degrees of cold acclimation; all of the plants were almost completely killed.

The high elevation coastal provenance (# 327) responded differently. After 4 weeks, the unfertilized cold room plants were much hardier and the difference was not apparent (possibly because of the poor replication in this one case) in plants conditioned for an extra week. Those grown in the shadehouse were more severely damaged on average than cold room stock; they showed an initial depression in hardiness under the proportionately higher potassium applications, followed one week later by a very distinct increase. In the shadehouse situation, these high elevation plants also exhibited an earlier preparation for winter in comparison with the 315 seed lot, whereas in the cold room they did not.

Levitt (1956) cites 21 papers showing that nitrogen decreases frost resistance, and only 5 that do not. Conversely, and by a similar balance of evidence, the effect of potassium was concluded to increase hardiness. However, none of Levitt's references refer to coniferous trees for which Pellet (1966) found little response to fall nitrogen applications.

More recently, Benzian and Freeman (1967) have found that if N and K dressings are applied to various conifers sufficiently late in the season, so that growth is not increased but nutrient content is, both elements, either separately or in combination, increased frost hardiness. This type of result could provide an explanation for the varied responses to fertilization in the present experiment, if it could be shown that growth responses (which oppose hardening) were induced in some cases and not in others, or if dormancy (and hence possible nutrient accumulation without growth) differed appropriately between provenances, as would be expected. In fact, although dry weight determinations were made, this was done after only 3 weeks and the data (not presented) show no consistent differences of an explanatory kind. It would be desirable to have further research examine this point with attention given to foliar nutrient contents, since not only might late season dressings increase cold hardiness, but Tukey and Meyer's data (1966) show that they can increase winter root growth considerably and provide an internal reservoir of ions for growth the following spring.

3.8 Foliar protein determinations

To provide sufficient material for reliable Kjeldahl nitrogen determinations, four freeze-dried replicates of a treatment were combined. Thus it was only possible to compare treatment means. The combined samples were ground and extracted four times with 80% ethanol for 5 minutes at 40 C to remove most of the free amino acids, guanadino compounds, ammonium salts, nitrates and other small N-containing molecules. The residue was presumed to contain largely the soluble and insoluble proteins with relatively small amounts of other N-containing substances such as purine and pyrimidine bases. The analysis was therefore only expected to reveal any obvious trends following the pattern of protoplasm augmentation and protein synthesis which Siminovitch and co-workers have observed (Siminovitch *et al.*, 1967; Pomeroy *et al.*, 1970). Figure 16 shows such trends to be absent among plants differing in hardiness. This analysis is regarded as inconclusive for want of more replicates (or clonal material) and more definite methods of protein separation.

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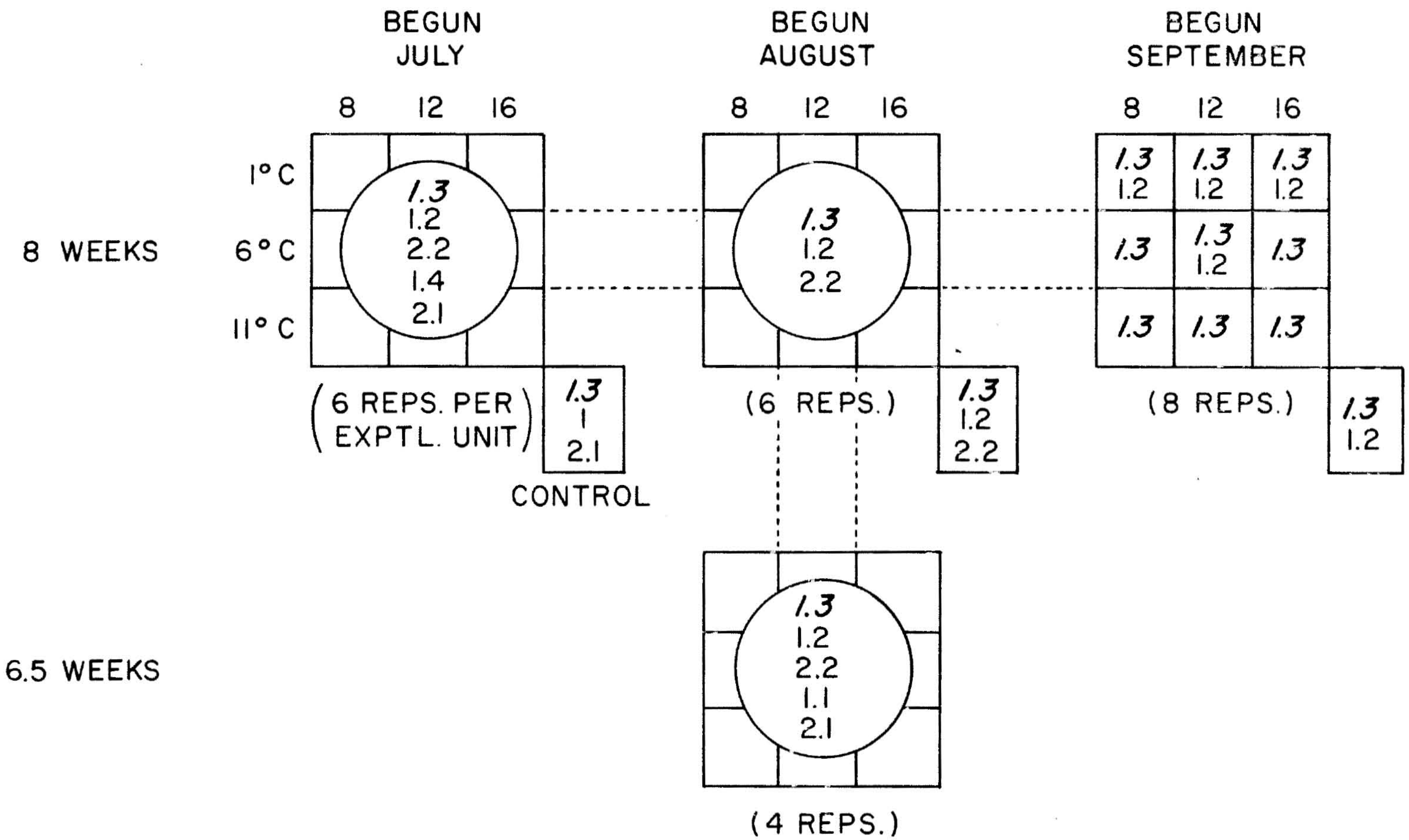


Figure 1. Allocation of plants to conditioning treatments in Expt. 1. (See page 3 for species and provenances corresponding to inscribed numbers).

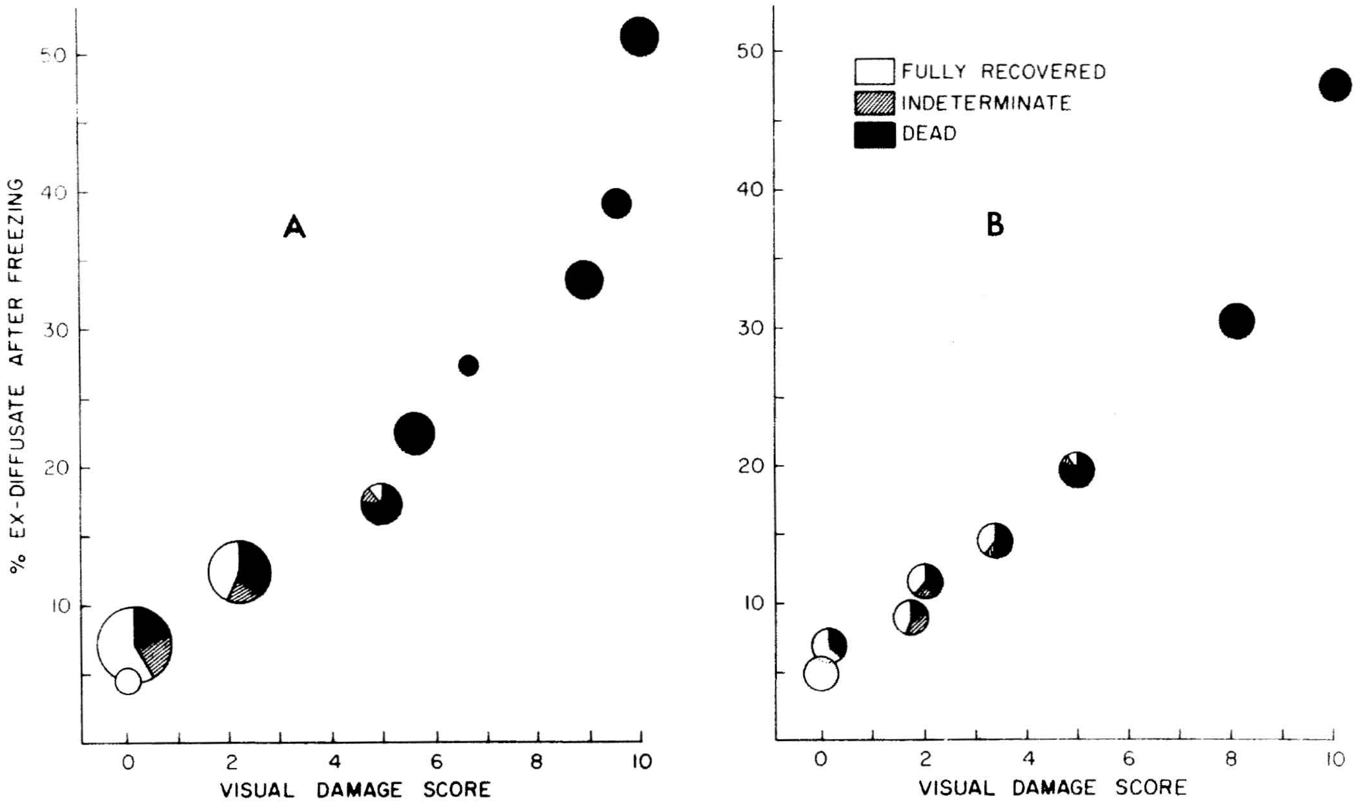


Figure 2. Relationship between conductivity and visual scoring methods for assessing survival of frost by coastal Douglas-fir seedlings:
(A) groups defined by 5% intervals across range of percentage conductivity values; (B) groups containing equal numbers of observations.

Notes: 1. Area of circle is proportional to number of observations contributing to the mean (varying from 2 to 33).

2. Shading of circle segments represent proportion of plants ultimately surviving.

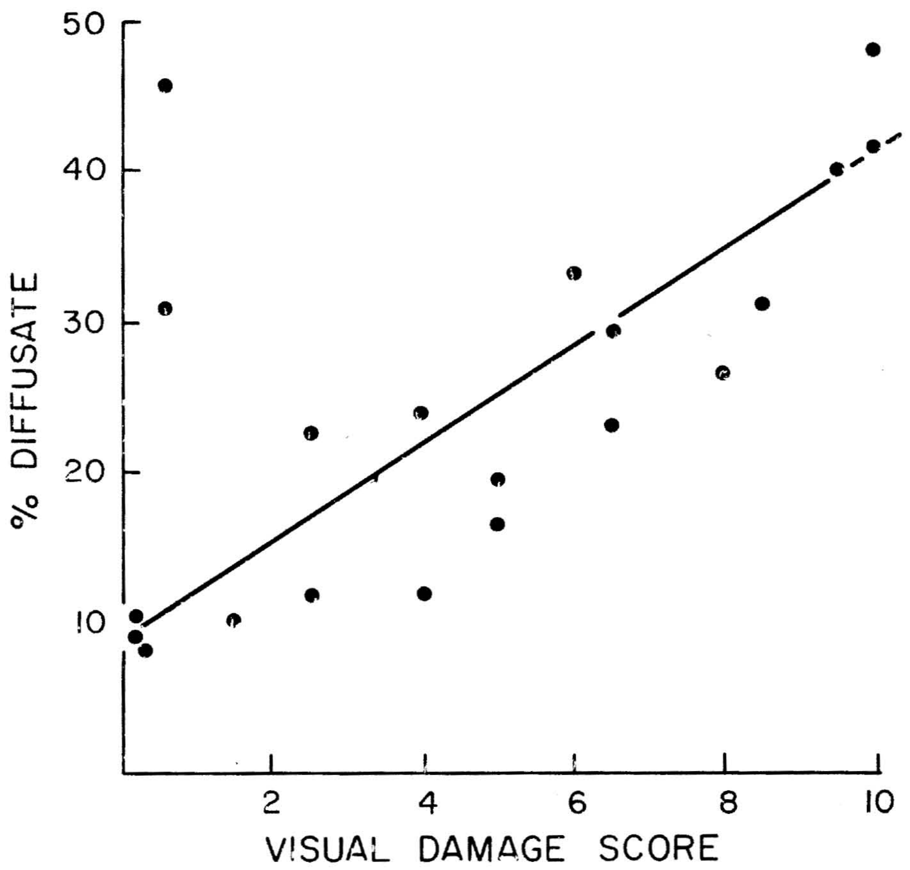


Figure 3. Relationship between conductivity and visual assessment methods for interior Douglas-fir.

Notes: 1. Plotted for -22C frost (-4, -8 and -12C provide inadequate damage score ranges).
2. Individual values, not means, are plotted.

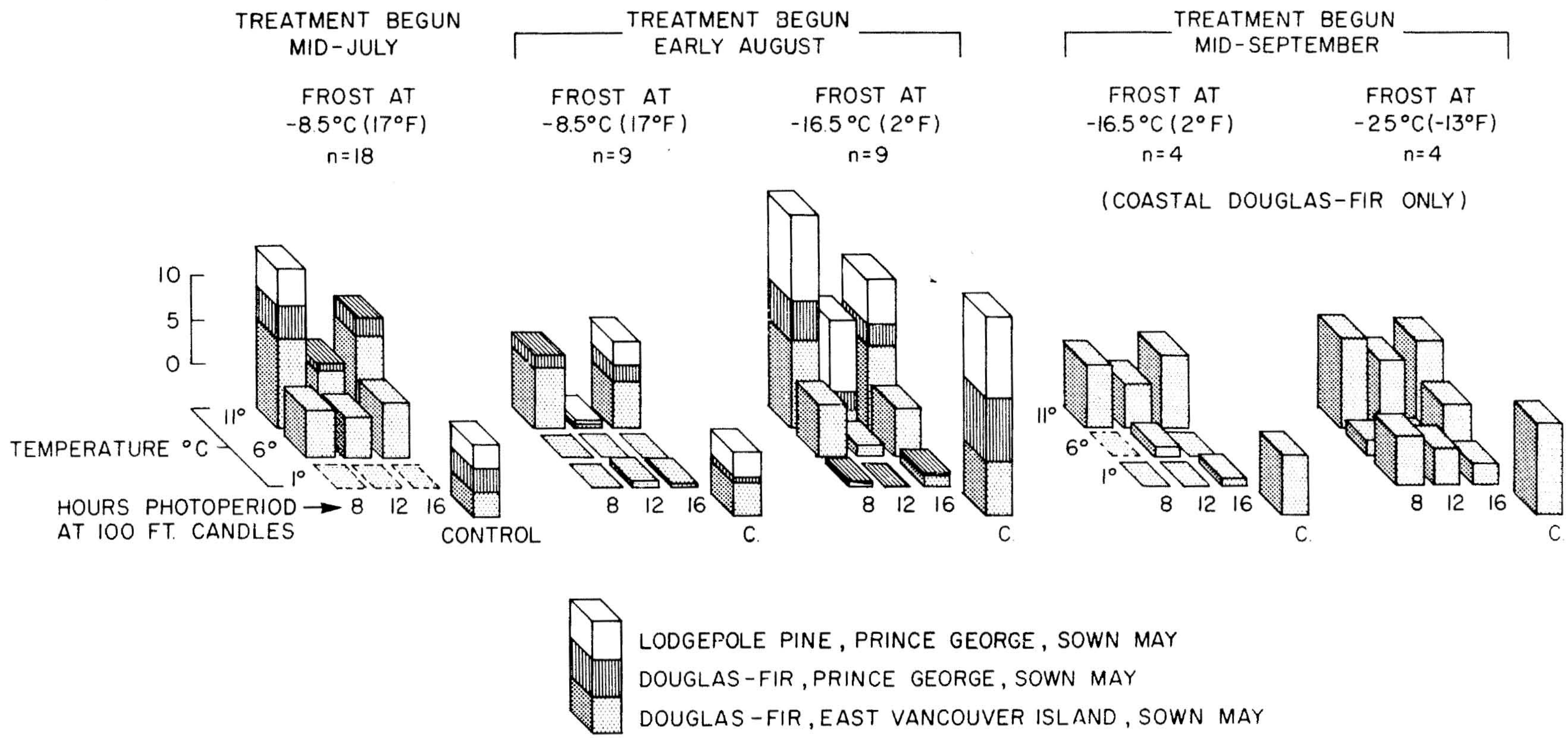


Figure 4. Damage caused by artificial frost to bullet-grown seedlings climatically conditioned for 8 weeks.

Notes: 1. Height of column represents damage to 'n' seedlings, n/3 of each species or provenance.
2. Each seedling scored visually from 0 (undamaged) to 10 (completely killed).

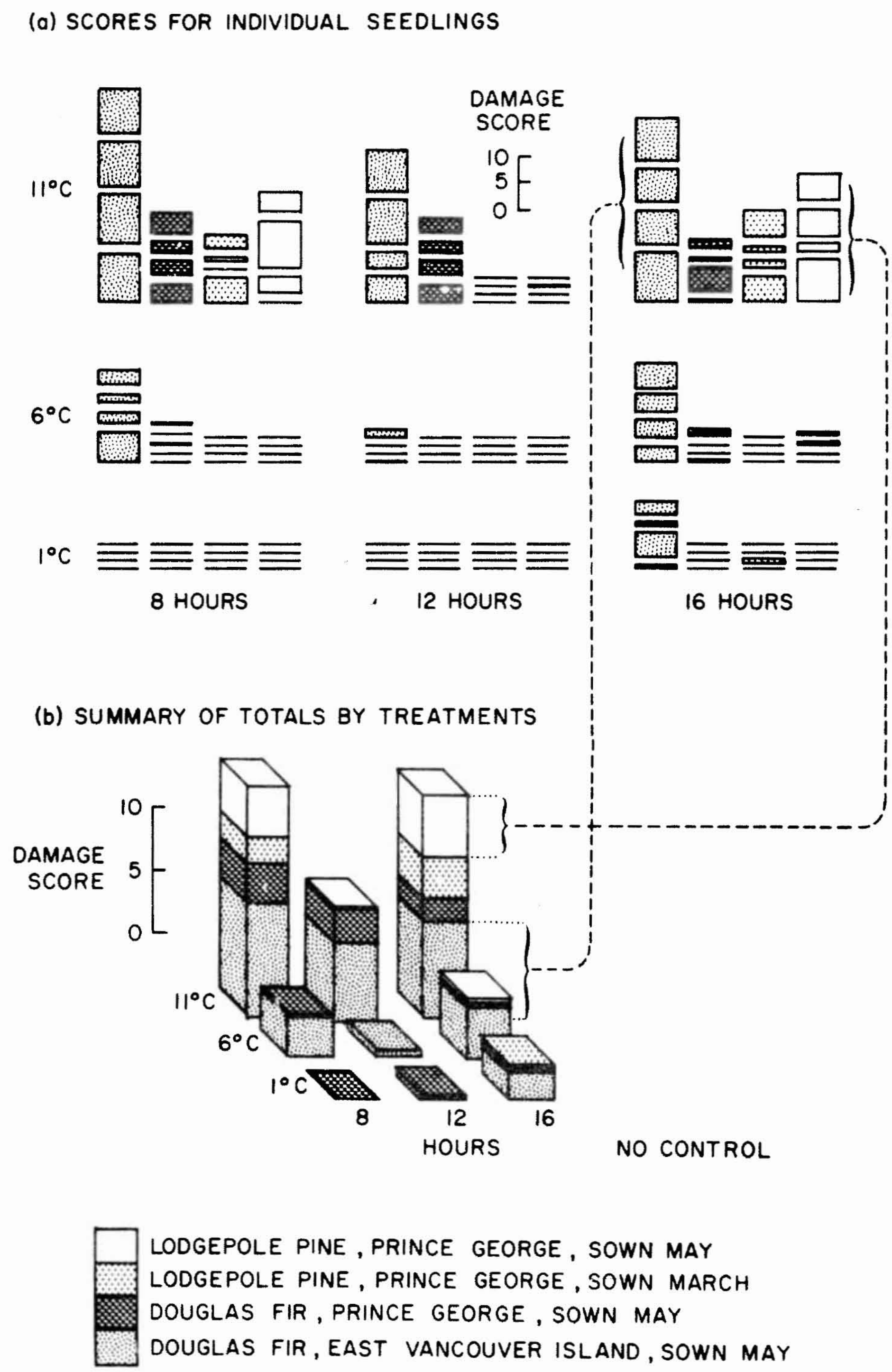


Figure 5. Damage caused by an artificial frost to bullet-grown seedlings climatically conditioned for 6 weeks; (a) illustrates variation within experimental units and construction of block diagrams, (b) treatment began early August with frost at -12C (10F).

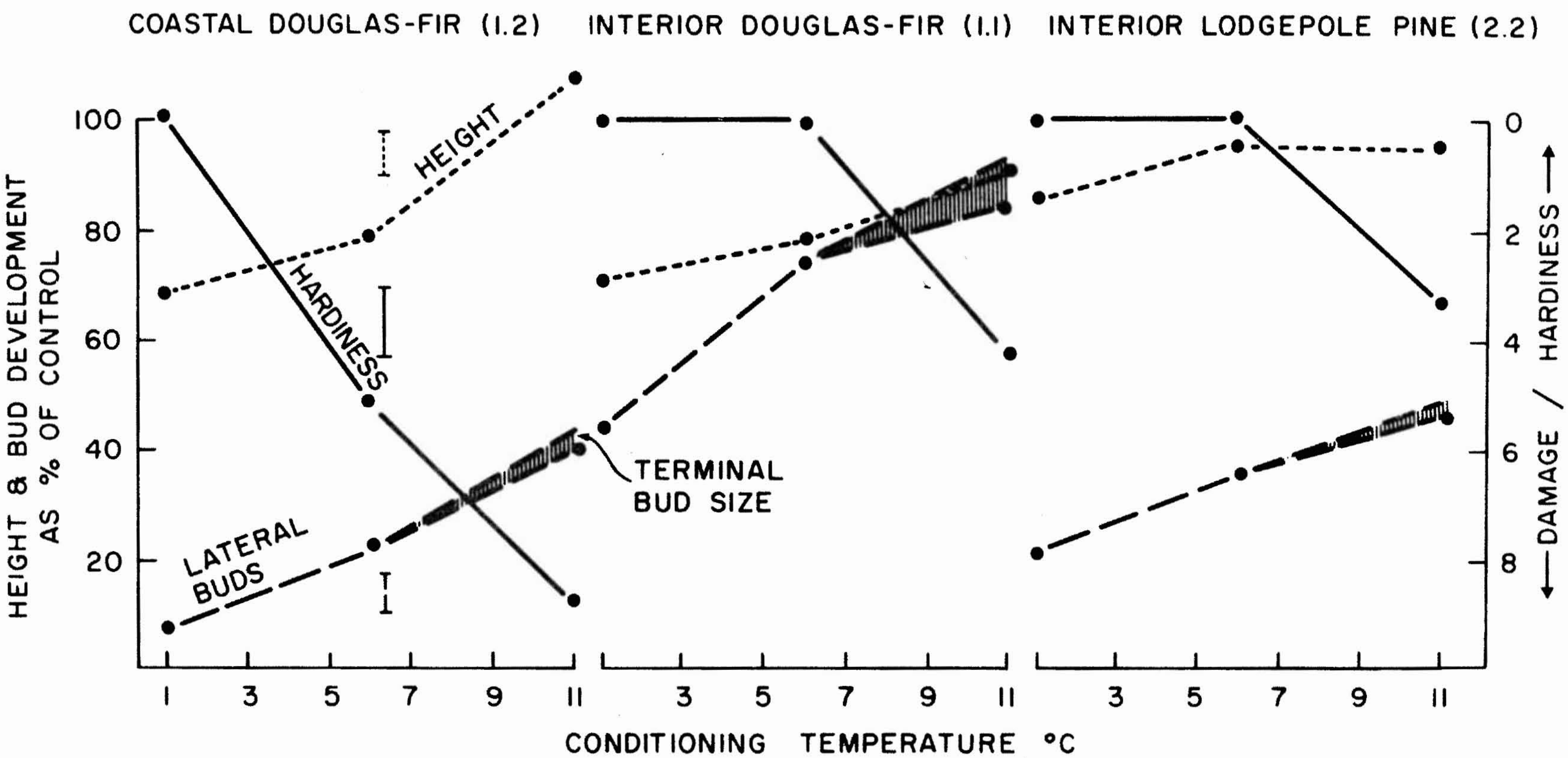


Figure 6. Height, bud development and hardiness relationships among the seedlings of Expt. 1. (Least significant differences at 5% probability level indicated, by vertical brackets, assuming common variance among species).

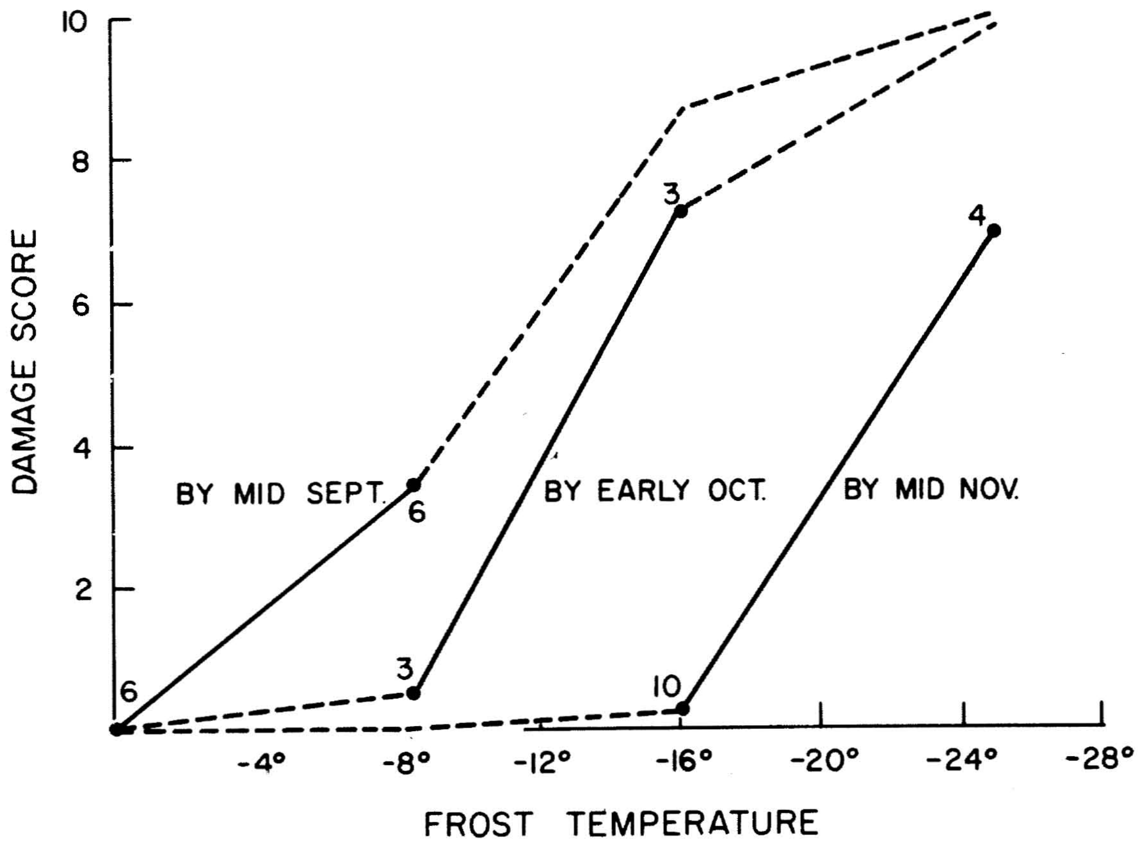


Figure 7. Natural hardening of controls of interior Douglas-fir in Expt. 1.

<u>Notes</u>: 1. Data taken from results of frosts on 'controls'.
2. Number by point denotes no. of observations contributing to the mean.
3. ---- indicates interpolated values.
4. The 3 curves differ significantly, as do any 2 measured points on a given curve.

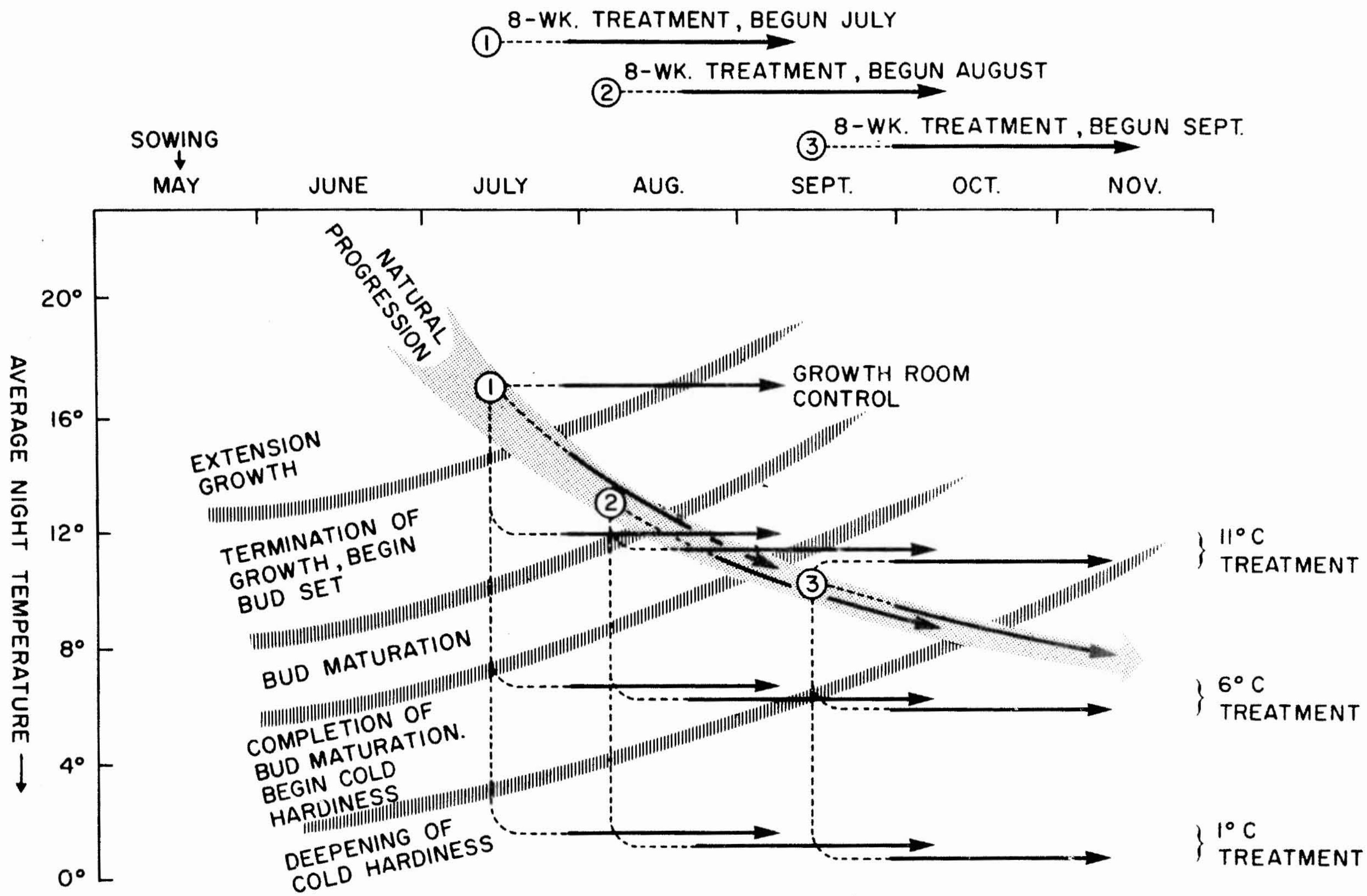


Figure 8. Schematic relationship of Douglas-fir seedling growth and hardiness to timing, duration and severity of cold treatments.

Notes: 1. Circled numbers indicate succession of timing treatments in their appropriate developmental positions at the beginning of the treatments.

2. Arrowheads indicate the growth status of plants at the end of their respective temperature treatments, or under natural outdoor conditions.

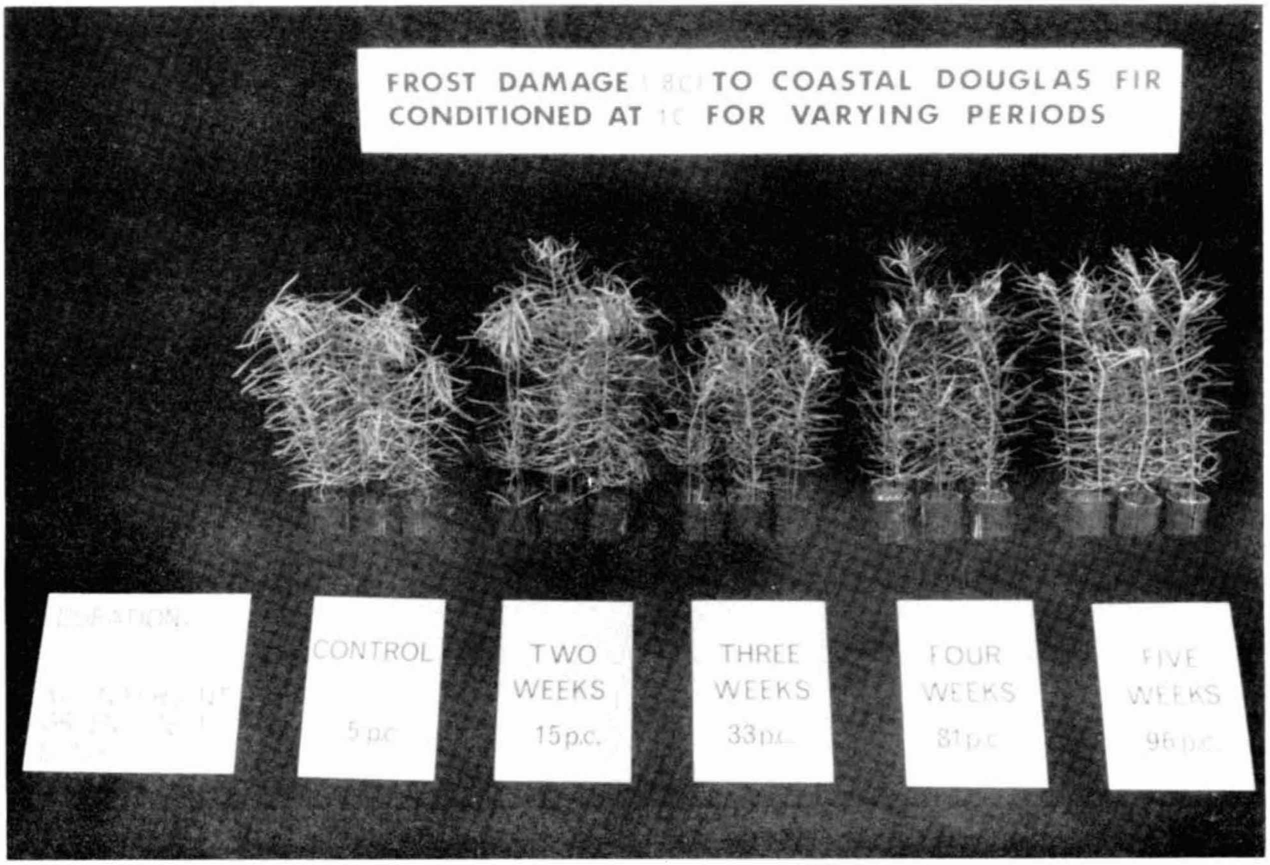


Figure 9. The effect of short acclimation periods on susceptibility of coastal Douglas-fir to light frost.

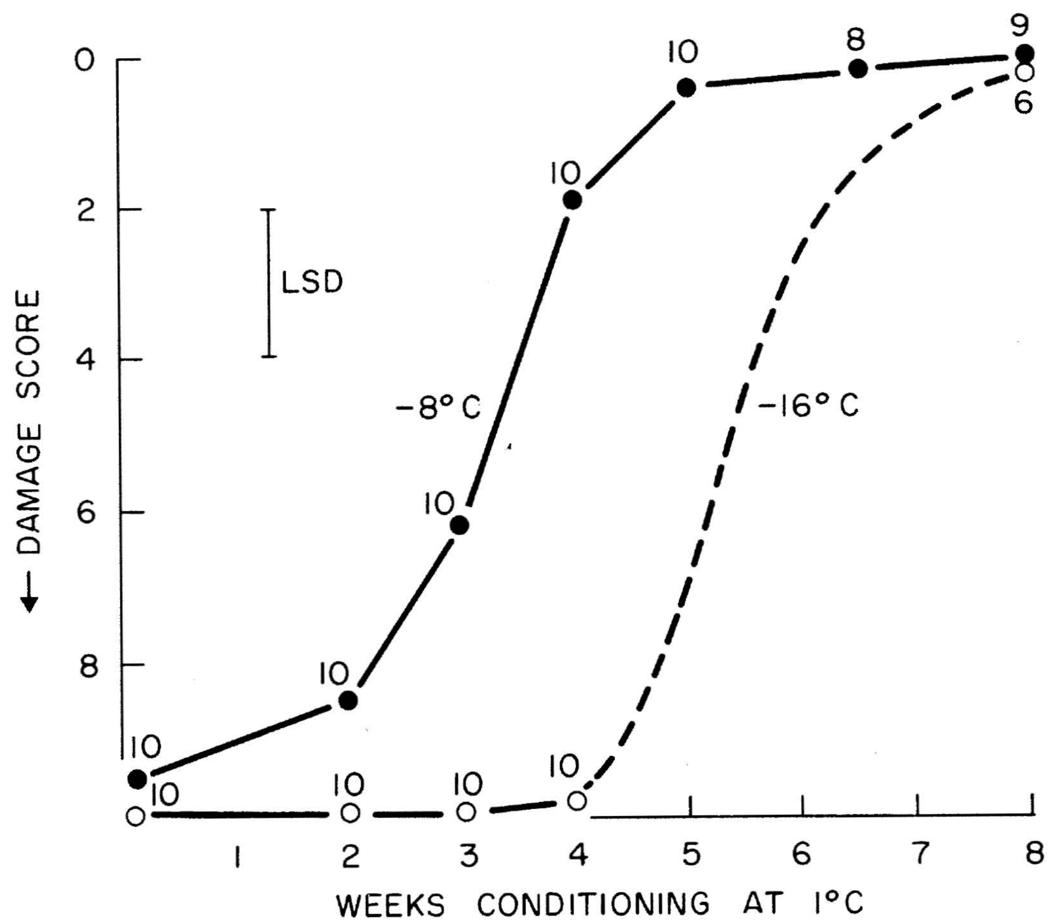


Figure 10. Effect of treatment duration on hardiness of coastal Douglas-fir.

Notes:
1. Numbers by points indicate observations contributing to mean.
2. 6.5 and 8-week treatments were on seedlings 1-2 months younger, under lower light intensity and unfertilized.
3. Least significant difference at 5% level is given.

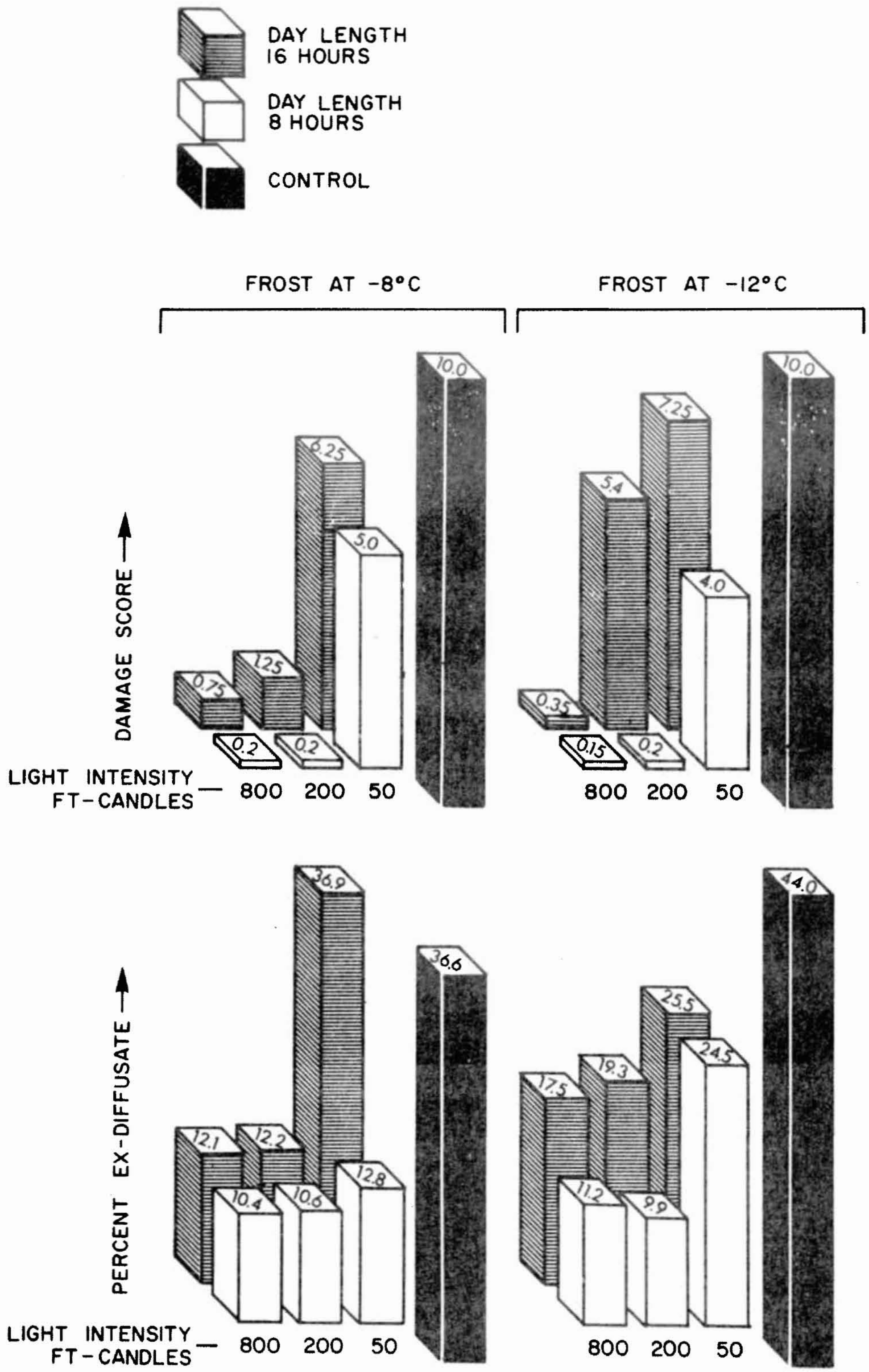


Figure 11. Effect of photoperiod and light intensity on hardiness of coastal Douglas-fir seedlings as measured according to two criteria and after two frosts.

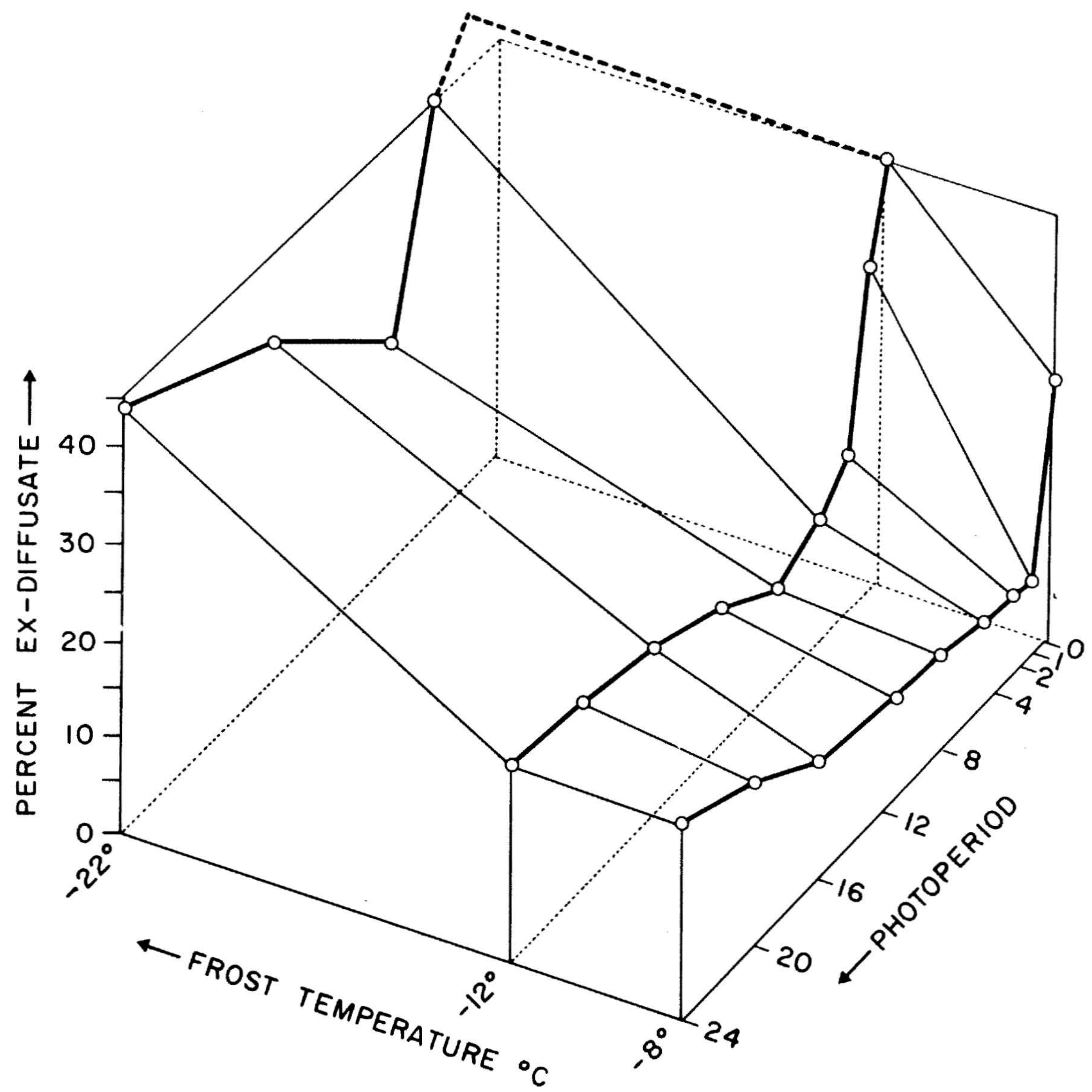


Figure 12. Frost damage to coastal Douglas-fir seedlings conditioned under a range of photoperiods.

Notes: 1. Each point is the mean of observations on 4 plants.
2. The increased hardiness at intermediate daylengths is highly significant.

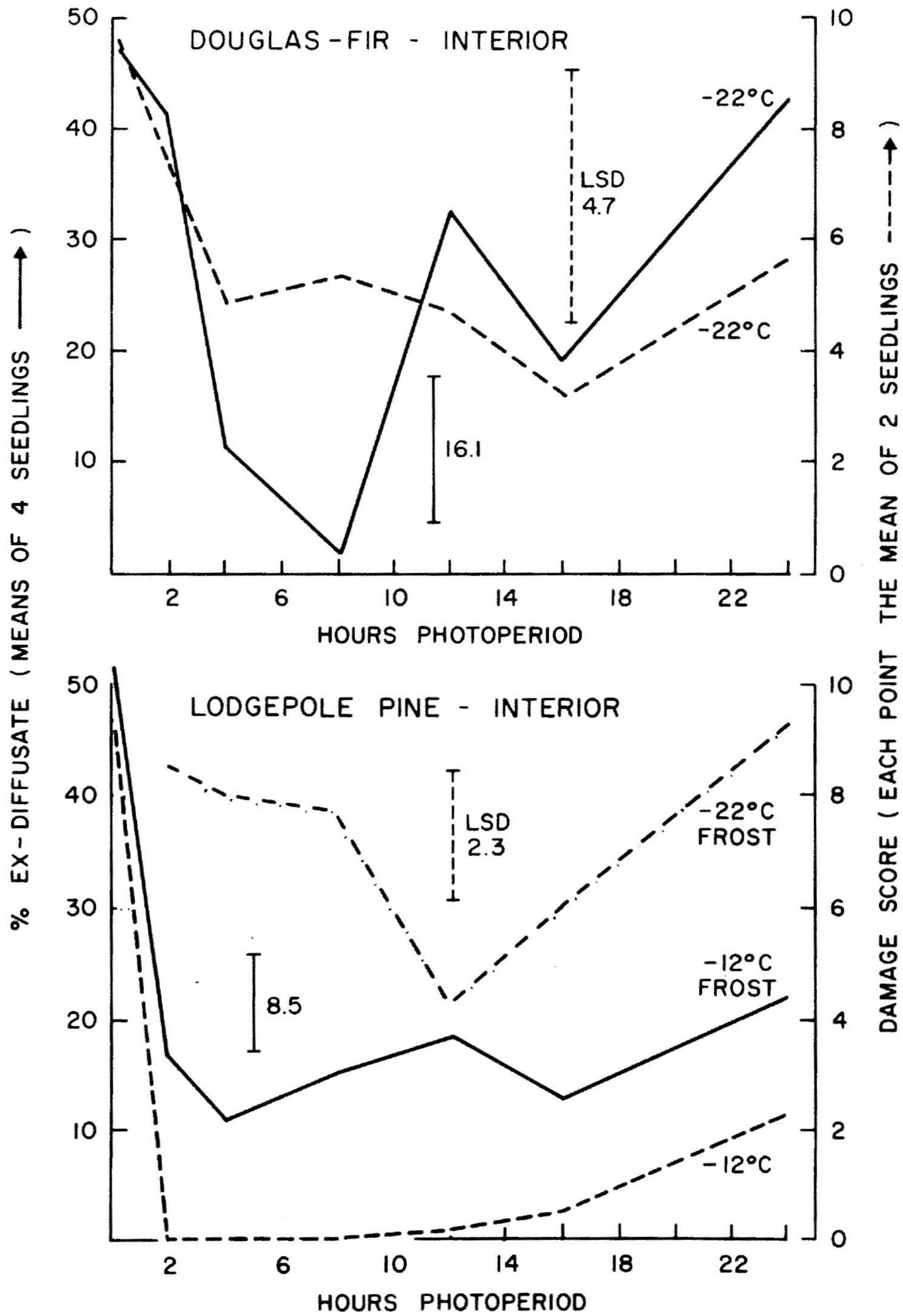


Figure 13. Relationship between cold hardiness and low temperatures for interior Douglas-fir and lodgepole pine seedlings after 6 weeks at 1C under a range of photoperiods. (Least significant difference at the 5% level is given.)

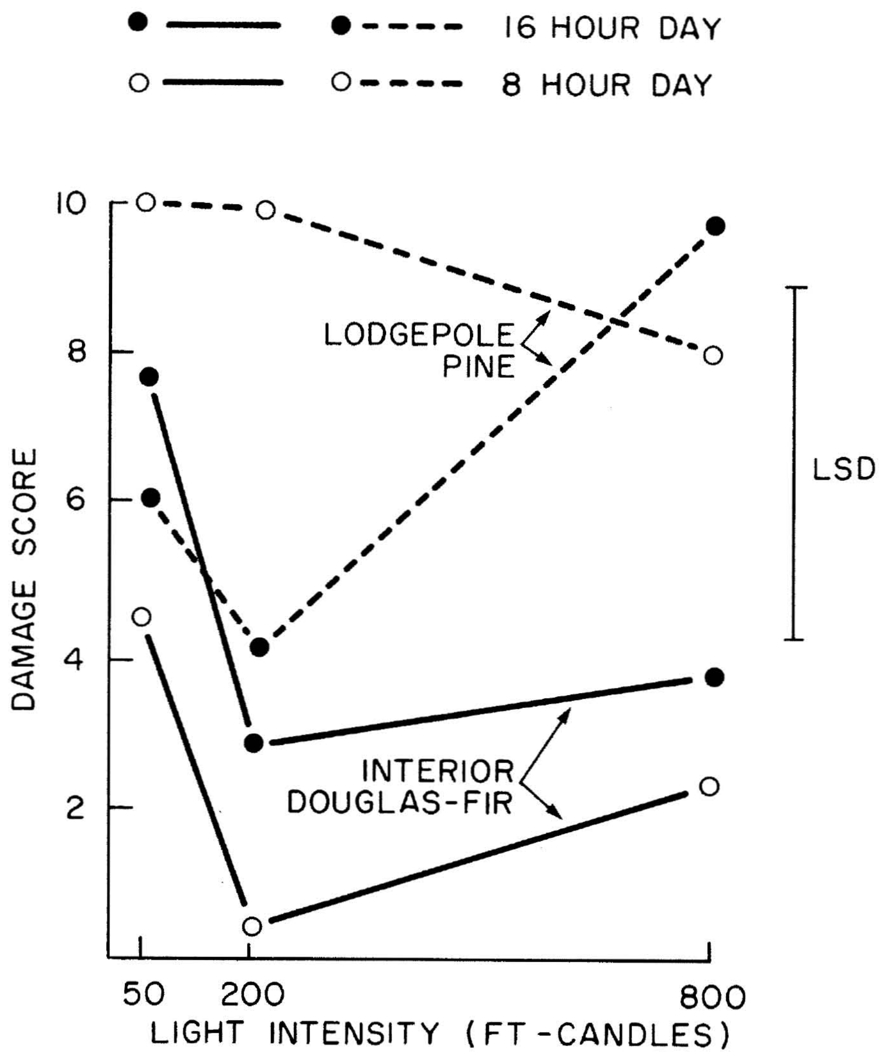
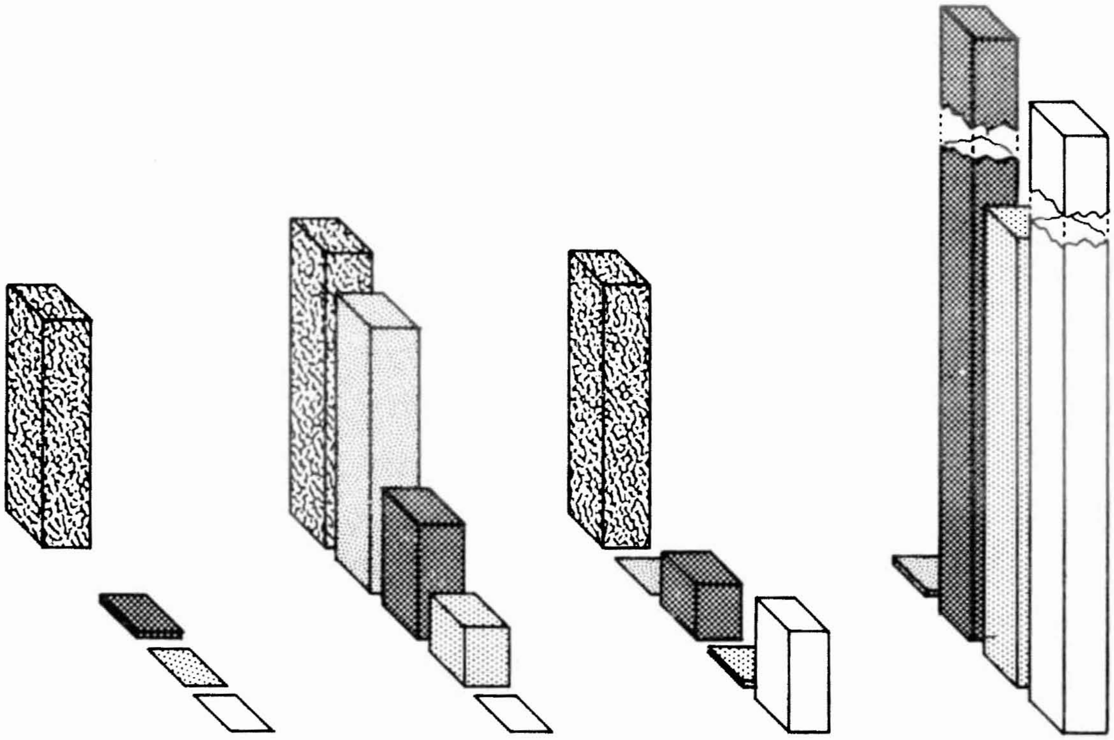


Figure 14. Frost damage to interior Douglas-fir and lodgepole pine seedlings after cold acclimation under various light regimes. (Least significant difference at the 5% level is given.)



MINIMUM WATER 550 FOOT-CANDLES

3.5	NOT TESTED	0.0	0	0
0.0		0.2	0	0
0.2		0.0	0	0
0.1		0.5	0	0
5.0		0.0	0	0
0.2		0.0	0	0
9.0		0.7	0	0

MINIMUM WATER 90 FOOT-CANDLES

4.0	5.0	0.1	0.2	0
1.0	0.0	1.0	1.5	0
0.1	3.0	1.5	0.2	0
0.0	2.5	0.2	0.0	0
5.0	2.0	1.5	0.2	0
1.5	0.0	0.2	0.2	0
11.6	10.7	4.5	2.3	0.0

DAILY WATER 90 FOOT-CANDLES

0.0	0	0.3	0.3	0.0
1.5	0	2.5	0.5	0.0
0.5	0	0.2	0.0	2.0
7.5	0	0.2	0.0	0.0
0.5	0	1.0	0.2	0.0
0.2	0	0.0	0.2	2.0
10.2	0.0	3.2	1.2	4.0

CONTROL, MOIST SOIL MAINTAINED

NOT TESTED	0.0	10.0	10.0	8.0
	0.0	8.0	5.5	9.0
	1.0	7.0	5.5	10.0
	0.0	10.0	1.5	9.0
	1.5	53.0	33.0	54.0

DOUGLAS-FIR, WEST VANCOUVER ISLAND, 2 GROWING SEASONS OLD (RAISED AT HANEY)
DOUGLAS-FIR, PRINCE GEORGE, SOWN MARCH (OF CURRENT GROWING SEASON)
DOUGLAS-FIR, EAST V. ISLAND, SOWN MAY (OF CURRENT GROWING SEASON)
DOUGLAS-FIR, PRINCE GEORGE, SOWN MAY (OF CURRENT GROWING SEASON)
LODGEPOLE PINE, PRINCE GEORGE, SOWN MAY (OF CURRENT GROWING SEASON)

Figure 15. Frost damage to seedlings conditioned under different light intensities and soil moisture regimes.

Notes: 1. The standard conditions were as follows:

a. Temperature: 5C (41F)
b. Photoperiod: 8 hours
c. Light quality: mixed fluorescent and incandescent
d. Duration: 8 weeks
e. 'Controls' grown under natural outdoor conditions, watered regularly and unfertilized.
f. Tested by artificial frost to -12C (10F).

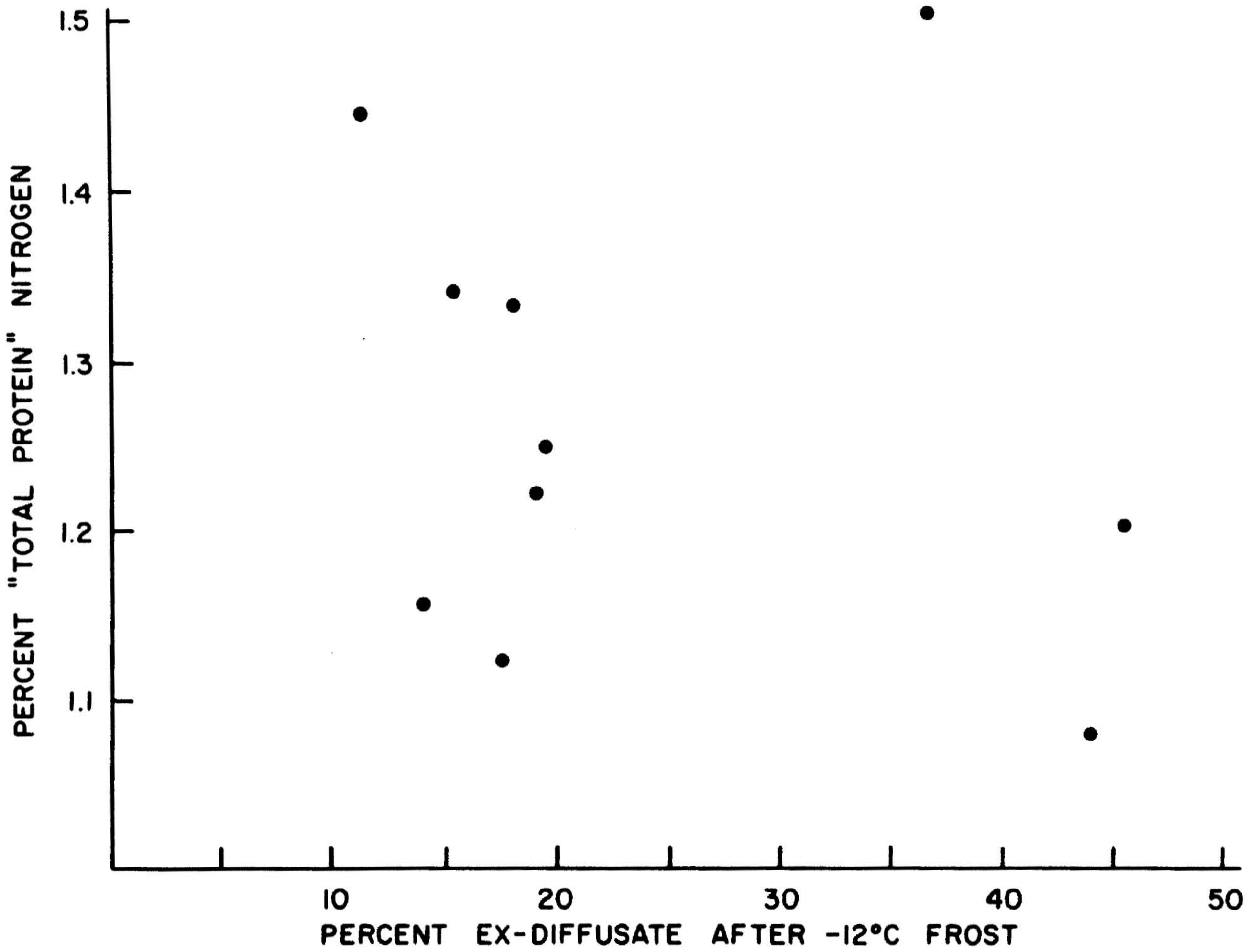


Figure 16. Relationship between "presumed total protein" nitrogen and frost hardiness of coastal Douglas-fir needles.