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# Frost hardiness in Scots pine (*Pinus silvestris* L.)

## II. Hardiness during winter and spring in young trees of different mineral nutrient status

*Frosthärdighet hos tall (Pinus silvestris* L.*)*

II. Härdighet under vinter och vår hos unga träd med olika mineralnäringsinnehåll

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# Abstract

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*Frost hardiness in Scots pine (Pinus silvestris L.) with different concentrations of mineral nutrients in the needles was determined during winter by different freezing treatments. For the determinations, needles from a fertiliser experiment in central Sweden were used. The main effect of fertilisation on nutrient concentrations in the needles was on nitrogen, which showed a marked responce to treatment. Frost hardiness determinations were made by measuring the leakage of electrolytes from the needles.*

*With freezing treatments that caused heavy damage, there was a maximum hardiness at nitrogen concentrations of between 1.3 to 1.8 % dw, whilst with moderate damage, no such maximum could be detected. In this case, a tendency towards decreasing hardiness with increasing nitrogen concentration was observed.*

*In the spring, 1971, two relatively hard frosts occurred in late May and early June. The frosts caused damage to several trees at many plots in the fertiliser experiment. The incidence of damaged trees increased above a needle concentration of about 1.8—2.0 % nitrogen dw, but even at high nitrogen content there were many plots with no damaged trees, suggesting that a factor or factors other than nitrogen content may influence frost hardiness. Frost hardiness did not appear to be related to needle concentrations of potassium. Low boron content, which has been associated with visual damage within the experimental area, was not directly related to hardiness as measured by freezing experiments.*

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# 1 Introduction

Winter damage to conifers occurs from time to time in Sweden. Damage is often limited to sites with a particularly hard climate (e.g. at high altitudes in northern Sweden) or with extreme climatic circumstances (Langlet, 1929). Injuries may also occur when trees are planted on sites with a more severe climate than that of their seed source, as observed in Swedish provenance experiments (cf. e.g. Eiche, 1966; Stefansson & Sinko, 1967; Kiellander, 1970a, 1970b).

This winter damage is often assumed to be caused by low temperatures. The damage is most often attributed to freezing (Langlet, 1929), but other factors such as drying out by frost (Bärring, 1967) or heavy loads of snow (Stefansson & Sinko, 1967), or a combination of frost damage and mechanical strangulation (Eiche, 1966) have been suggested as possible causes.

The concentration of mineral nutrients within the plant markedly influences frost hardiness. The Department of Forest Ecology has set up a series of extensive fertiliser experiments over the past 20 years, the main aim of which is to study the influence of different fertiliser regimes on the growth of the two native coniferous species (Scots pine and Norway spruce), under different site conditions. The influence of fertilisation on aspects of tree development is also being studied.

The aim of the present work was to investigate the effect of mineral nutrition on frost hardiness. In the investigation of frost hardiness, plant parts were taken from one of the fertiliser experiments at Lisselbo. The experimental area is situated on the west slope of a sandy eskar at latitude 60° 28′ and longitude 16° 57′ E, at about 80 m above sea level. The whole area is rath-

Table 1. Fertilisation treatments at the Lisselbo experimental site from 1969 onwards. Treatments with different elements were applied to the experimental plots according to the plan in Figure 1. Elements in kg per ha för columns N1—Ca. N as ammonium nitrate, P as triplesuperphosphate, K as potassium chloride, Mg as magnesium carbonate, S as sodium sulphate, Ca as ground limestone. Micro was a mixture of micronutrients. Acid was given as kg $H_2SO_4$ per ha.

| Year | Treatments | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N1 | N2 | N3 | P2 | K2 | Mg | S | Ca | Micro | Acid 1 | Acid 2 |
| 1969 | 60 | 120 | 180 | 40 | 76* | 50 | 40 | 1960 | | | 100 |
| 1970 | 60 | 120 | 180 | | | | | | + | 50 | 50 |
| 1971 | 40 | 80 | 120 | | | | | | | 50 | 50 + 50 |
| 1972 | 40 | 80 | 120 | 20 | 38** | | | | | 50 | 50 + 50 |
| 1973 | 40 | 80 | 120 | | | 50 | 40 | | + | 50 | 50 + 50 |
| 1974 | 40 | 80 | 120 | | | | | | | 50 | 50 + 50 |
| 1975 | 30 | 60 | 90 | 40 | 78* | | | | | 50 | 100 |
| 1976 | 30 | 60 | 90 | | | | | | | 50 | 100 |

* 80 kg in experiment E41.
** 40 kg in experiment E41.

er flat and the height difference between the top of the eskar and its surroundings is no more than five to ten metres. The former pine stand on the area was destroyed by a storm in early 1954. About six or seven seed trees per ha were left after the harvest caused by the storm. During the summer, 1955, the area was scarified and the patches were sown with seeds from latitude 61°, 0—150 m above sea level. Stand thinning was carried out in 1961 and 1965.

Three different experiments (designated E40 to E42) were laid out within the area (Figure 1). E40 is a nitrogen fertiliser experiment with three different dosages. E41 is an experiment with blocks to test the effect of adding one or more of the elements, phosphorus, potassium, magnesium and sulphur (and also in a few cases, the micronutrients, copper, zink and boron) to plots receiving nitrogen at the medium dosage for E40. E42 is a mixed experiment with acidification, liming and irrigation. The fertiliser regimes from 1969 to 1976 are given in Table 1. The effects of the treatments are followed continually by tree growth measurements (height and diameter). The nutritional status of the stand is studied by sampling exposed current needles each autumn and analysing them for plant nutrients.

For more information about the experimental area and the treatments, see Tamm et al., 1974.

Abbreviations: For the mineral nutrients, chemical symbols are used. RC (Relative Conductivity) is the conductivity of a water extract from the frozen material, expressed as a percentage conductivity of the same extract after the material has been killed by boiling.

# 2 Methods

In an earlier investigation (Aronsson & Eliasson, 1970) it was shown that there is a positive relationship between the frost hardiness of tree stems, needles, and buds. Since needles make up more than half of the shoot mass and are easy to work with, they were used for frost hardiness tests.

Analysis of mineral nutrient content of the needles was done as part of the department's routine work in the fertiliser experiments. For these analyses, needles from exposed current shoots from the second whorl were collected during October. Ten trees from each plot were used and a bulk sample from each plot was analysed for N, P, K, Ca, Mg, and Mn on a dry weight basis (Ingestad, 1979). In some cases, B, Cu and Zn were also analysed.

For testing frost hardiness, samples were taken on 23rd of January and 14th of March, 1971; 5th of February and 21st of March, 1972. Exposed second order shoots from the third (and in a few cases the fourth) whorl were taken. Ten trees (as a rule the same trees as for mineral nutrient analysis) from each plot were used. The plots used are indicated with an asterisk in Figure 1.

Because the available freezer space was limited and large amounts of plant material in the freezer are associated with long time intervals to achieve a desired temperature, each shoot was divided into five pieces and only one piece was used at each test.

All the pieces from a plot were thereafter divided into five subsamples in such a way that each subsample of ten pieces contained two basal pieces, two basal-adjacent pieces, two middle pieces and so on (Figure 2). The reason for this arrangement was to minimise errors from a possible difference in frost hardiness of needles according to their position on the shoot. Before the investigation started, a few tests were made to determine whether such a gradient existed. Although no systematic differences in frost hardiness among different parts of the shoot were detected, it was decided to maintain the sampling procedure.

After the samples had been divided, the material was stored in a well insulated box and transported to the laboratory for frost hardiness determinations. One of the subsamples was immediately tested while the others were carefully insulated in a large number of paper bags and stored in a deep-freeze at —12°C for testing at a later date.

In earlier experiments it had been possible to collect all plant material immediately prior to the freezing tests. In this investigation, it was considered desirable to test the effect to storage on the measurements. Therefore, some shoots from the first sampling of 1971-01-23 were stored at —12°C for a little more than one month.

The determinations of frost hardiness were carried out as described by Aronsson & Eliasson (1970). In brief, the method is as follows. The test material is stored in plastic bags in a refrigerator at 4°C and left for temperature equilibration for 18 hours before the freezing treatment. Freezing is done by placing the plant material in deep-freezes at preset temperatures. By this method it is possible to obtain differences in tissue damage even in material of considerable hardiness, such as Swedish pine provenances sampled in winter, which probably would survive the temperature of liquid air if cooled very slowly (Tumanov & Krasavtsev, 1959; Weiser, 1970). During freezing, all the material from all the plots was carefully mixed and spread out on a net frame in the deep-freeze (Figure 2). To minimise supercooling, each deep-freeze was equipped with a fan. After six hours in the

E40 - E42 LISSELBO

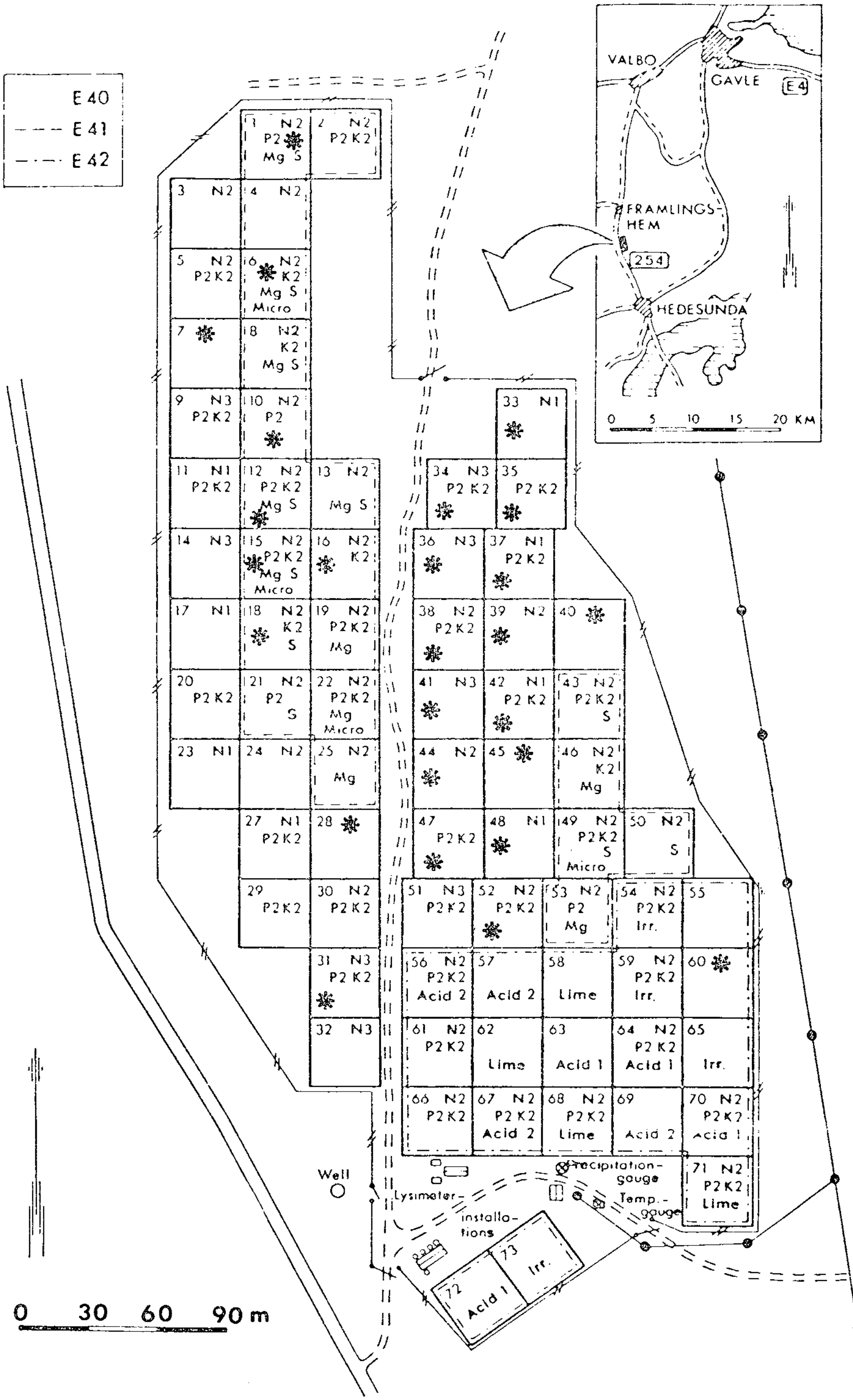



Figure 1. The experimental plan of the Lisselbo site. The plots comprise three different experiments, E40, E41 and E42. Plots used for frost hardiness determinations during the winters of 1971 and 1972 are marked with an asterisk.

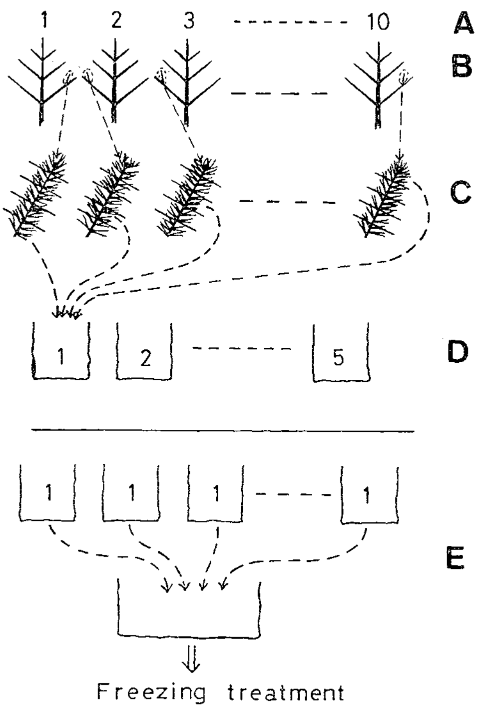



Figure 2. Scheme for dividing the sampled shoots from any plot into subsamples. A: From each plot 10 trees were selected. B: Second order shoots from the third or fourth whorl were sampled. C: The shoots were divided into five pieces and each piece marked. D: Each piece was divided into a further five subsamples as indicated. E: The subsamples from all plots, used in one frost hardiness determination, were carefully mixed before the freezing treatment.

freezer, the material was thawed in a refrigerator for 18—20 hours. The test temperatures were —12°C ±1°.5, —22°C ±1°.5, —32°C ±1°.5 and —44°C ±2°.0.

Damage caused by freezing was estimated by conductivity measurements on water extracts of the needles, according to the method described by Aronsson & Eliasson (1970). For extraction, distilled water was added to the sample (0.5 g) in the weight ratio of 20 parts water to one part sample and was shaken for 18—20 hours at room temperature. The conductivity of the water ($\varkappa_{\text{frozen}}$) was then measured at 25°C. The tissues were then killed by means of boiling the plant material for 10 minutes and the extract was shaken for a further 18—20 hours. A new conductivity measurement ($\varkappa_{\text{boiled}}$) was carried out. Relative conductivity (RC) was calculated as follows:

$$RC = \frac{\varkappa_{\text{frozen}}}{\varkappa_{\text{boiled}}} \times 100$$

Low RC-values indicate that the plants had suffered little or no damage during freezing. High values indicate that the tissues were severely damaged or killed.

On the first sampling occasion, some of the freezing treatments were repeated several times (Figure 6). These repeated freezings were standardised, i.e. the material was transferred directly from a refrigerator at a temperature of 2—4°C into the deep-freeze, at a preset test temperature. The freezing period was six hours, and between each freezing treatment the shoot pieces were allowed to thaw in a refrigerator for 18 hours.

Within the field experimental area, a meteorological cage (Figure 1) was placed, in which air temperature and humidity were recorded.

A freezing experiment was carried out in the field on the night between 8th and 9th of June, 1972, when shoots on standing trees at Lisselbo were artificially frozen. The equipment consisted of a cooling unit with a freezing mixture of liquid water and glycol, which was pumped through several cylindrical coils of copper tubing, connected to the freezing bath through insulated plastic tubes. The shoot was placed inside the copper cylinder and the temperature was monitored by two thermistors attached firmly to the bark of the shoot. The freezing treatment lasted between half-an-hour and one hour.

# 3 Results

### 3.1 Storage of material

For the sampling occasion, 71-01-23, frost hardiness increased during storage for 37 days in the deep-freeze at —12°C (Figure 3). On the later sampling occasions no such hardening occurred. Before the sampling date, 71-01-23, the minimum temperatures had been above 0°C for five days, whilst at the other sampling times the minimum temperatures had been at subzero levels (Figure 4). It seems reasonable to ascribe the observed differences in deep-freeze hardening of the material from different dates to the daily temperatures prior to each sampling occasion. No effect of needle nitrogen content on storage response could be detected.

### 3.2 Winter frost hardiness and damage

Frost hardiness measurements on individual trees on the sampling occasion, 1971-01-23, show rather big differences between trees within the same plot (Figure 5). With greater damage, the differences between the trees increased. Repeated freezings (Figure 6) caused increased damage, although three

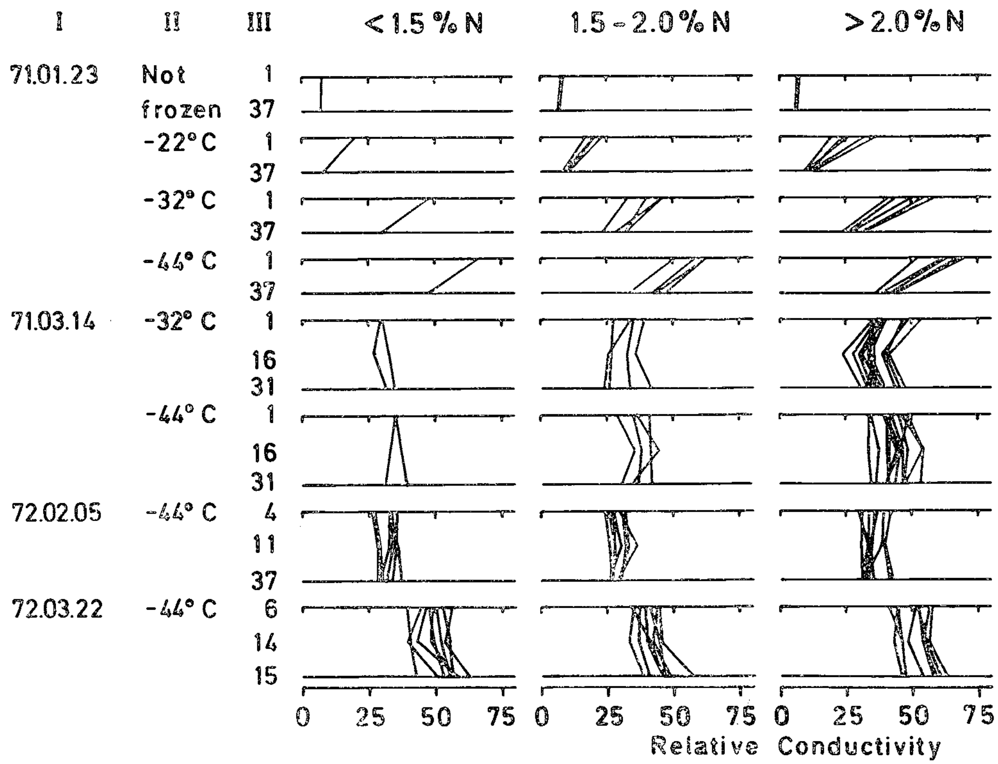



Figure 3. The effect of storage period on shoot sample frost hardiness. Column I refers to sampling date, column II to freezing treatment, and column III to the number of days the shoots were stored at —12°C between sampling and frost hardiness determinations. Results are given for three different classes of N-content of needles.

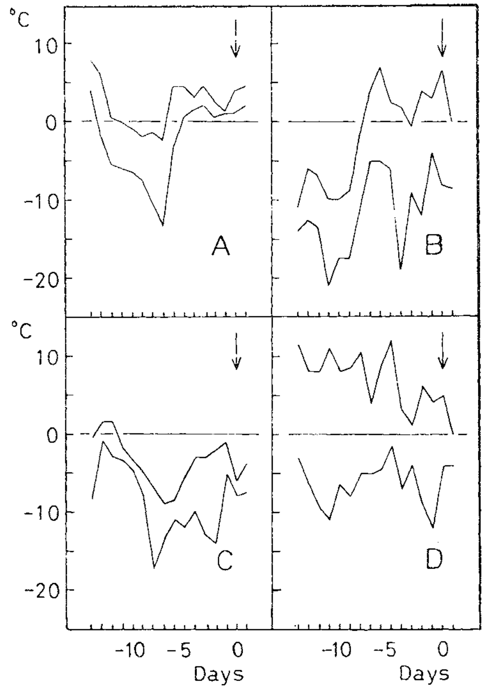



Figure 4. Daily minimum and maximum temperatures prior to the sampling dates; day 0 also indicated by arrows. Sampling dates: A, 1971-01-23; B, 1971-03-14; C, 1972-02-05; D, 1972-03-21.

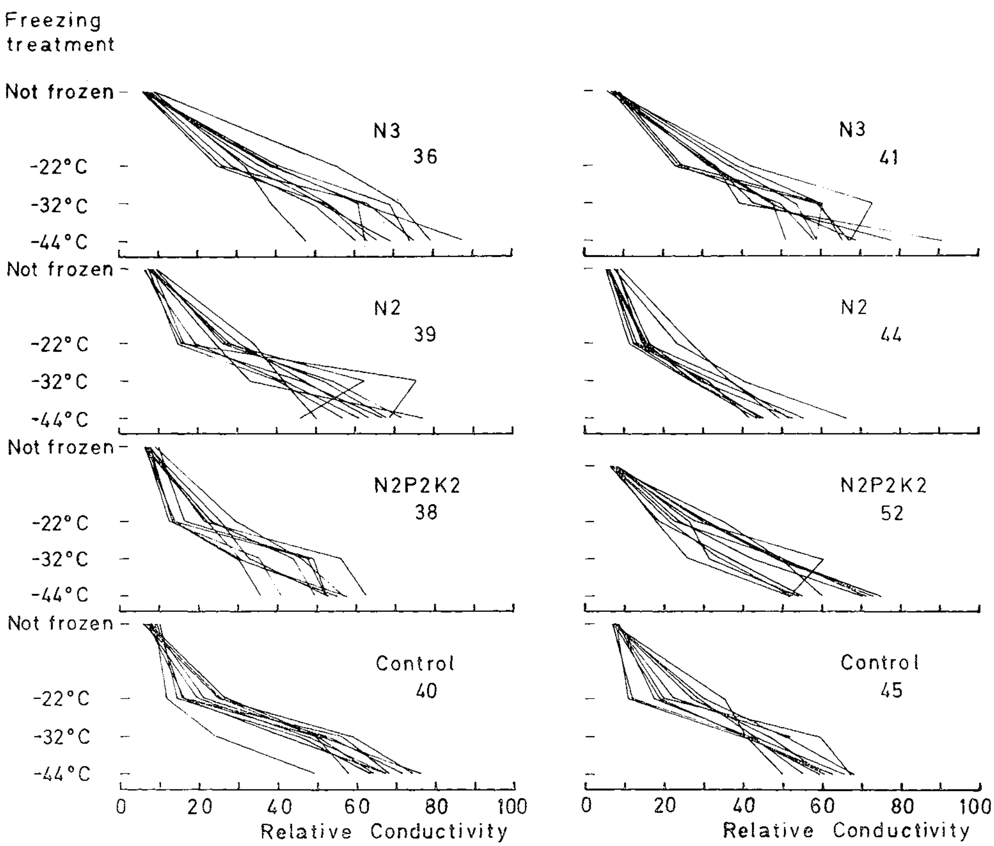



Figure 5. Frost hardiness for individual trees at the sampling date, 1971-01-23. Fertilising treatment and plot number are indicated within the sub-figures. For details of treatments, see Table 1 and Figure 1.

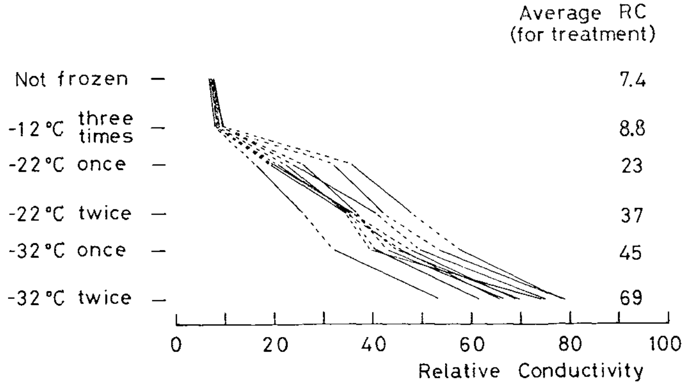



Figure 6. Damage caused by one (or more) freezing treatment of samples from 10 different plots, 1971-01-23. Each line connects data for subsamples from the same shoots (cf. Figure 2). From each plot ten trees were sampled. Range in N-concentrations 1.54—2.96 % dw.

times to —12°C caused only very small damage. Increasing needle damage was observed with lower freezing temperatures and more than one freezing treatment, with the greatest damage at two freezing occasions to —32°C.

In Figures 7 to 9 damage on each sampling occasion in the winters of 1971 and 1972 is shown as a function of the N-content of the needles. The tests on 1971 material (Figure 7) indicated that at higher N-content, frost hardiness decreased.

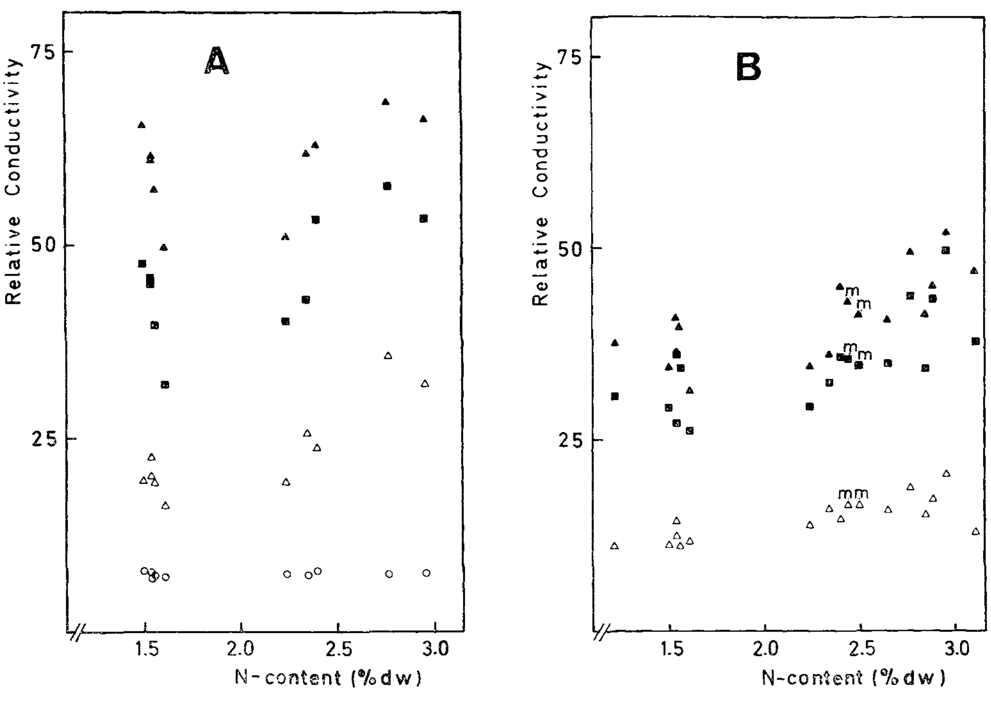



Figure 7. Variation in frost hardiness at different needle contents of nitrogen. Each sign is for one plot. Ten trees were used from each plot. In subfigure A (and in B for —22°C) the values are from one determination, whilst in subfigure B, the values for —32° and —44°C are mean values of three determinations. Sampling date: A, 1971-01-23; B, 1971-03-14. Symbols: ○ not frozen(control), △ frozen to —22°C, ■ frozen to —32° C, and ▲ frozen to —44°C. "m" refers to the plots fertilised with a micronutrient mixture of copper, zinc and boron.

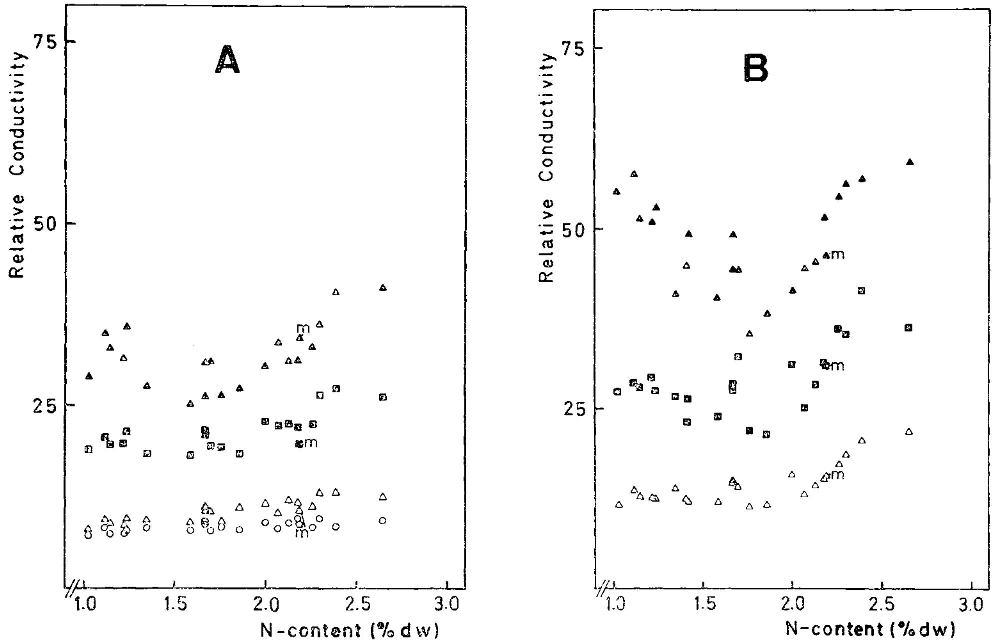



Figure 8. Variation in frost hardiness at different contents of nitrogen in exposed current needles. Each sign is for one plot. The values for not frozen, —22°C, and —32°C are from one determination each, whilst the values for —44°C are means of three determinations. Sampling dates: A, 1972-02-05; B, 1972-03-21. Symbols as in Figure 7.

However, there was a rather big gap in N-contents with no data points for N-content between 1.6 and 2.2 % and only a few plots with a N-content lower than 1.5 %. One could not, from this data, exclude the possibility that frost hardiness had a maximum (which means that the RC-values had a minimum) within this range. The number of samples was therefore increased in 1972 and chosen in such a way as to minimise the interval in nitrogen contents. These data are shown in Figure 8 and seem to indicate that there is indeed a maximum in frost hardiness at about 1.5—1.7 % N in the needles.

The values in Figure 8 were tested to determine how well they fitted the following equations:

$$RC = N^2 + c,$$
$$RC = N^2 + bN + c,$$
$$RC = bN + c.$$

Of the equations, $RC = N^2 + bN + c$ gave the best fit for almost all the freezing treatments (Figure 9). One of the —22°-freezings, which showed very little damage, fitted better to a straight line, as did also the control. Minima in the RC-values increased from about 1.3 % N at slight damage to about 1.8 % N for very heavy damage.

At the time of sampling in the winter of 1971, the plots had received two applications of N fertiliser and, in 1972, three applications, but only a single addition of potassium and phosphate (Table 1). The treatments had resulted in strongly increased nitrogen concentrations in the needles, whilst the concentrations of other elements had been much less affected (Figure 10). Because of the high nitrogen concentrations in 1970, the applications in 1971 were decreased, which resulted in a fall in nitrogen concentrations for that season. The concentrations of other elements in the needles were negatively related to those of nitrogen (Table 2, Figure 10). For some plots, Cu, Zn and B have also

Table 2. Correlation coefficients for concentrations of mineral nutrients in exposed current needles for the 21 plots sampled on the 5th of February, 1972. Sampling for mineral analysis was done in October, 1971. Levels of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

| | P | K | Ca | Mg | Mn |
|---|---|---|---|---|---|
| N | —0.57** | —0.57** | —0.54* | —0.61** | —0.51* |
| P | | 0.51* | 0.24 | 0.22 | 0.43 |
| K | | | 0.08 | 0.31 | 0.58** |
| Ca | | | | 0.69*** | 0.54* |
| Mg | | | | | 0.62** |

been analysed for different years between 1968 and 1976. There is a negative relationship between needle concentrations of N and B, except for those plots that had received B. In such cases, the B content was comparatively high. For Zn and Cu there were no changes in concentration, irrespective of the fertiliser treatment.

During the autumn, 1974, an investigation was made on visibly damaged trees from all Lisselbo plots, from which a relationship between B-content in the needles and the percentage of damaged trees per plot (Figure 11) may be seen.

### 3.3 **Spring frost damage, 1971**

At the beginning of the 1971 growing season, two relatively hard periods of frost occurred, which were recorded in the meteorological cage (Figure 1). The temperature measured inside this cage represents air temperature. During nights with no wind, needles on exposed branches might well have had night temperatures one degree or more lower than was recorded.

From the 22nd to the 24th of May, three nights occurred during which relatively long periods of low temperature (—4.0, —3.5, and —7.5°C) were recorded (Figure 12). A second period with low night temperatures occurred between the 6th and 14th of June. During this period, there were four nights with temperatures below zero. The first and last of these four nights had only light frosts (—2.0°C or above) and it seems un-

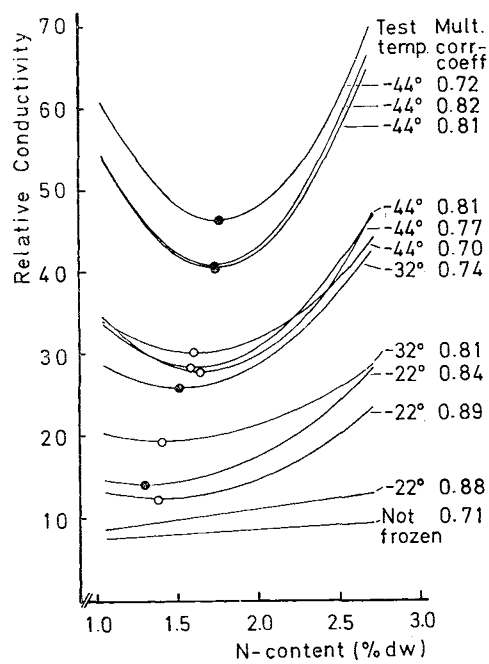



Figure 9. Curves for the different freezings during 1972 fitted to the equation $y = x^2 + bx + c$, where $y$ = RC-value, and $x$ = N-content of the needles (% of dry weight). For the —22°C-freezing at the bottom of the figure and for "Not frozen", the values fitted better to a straight line than to the equation used for the other freezing treatments. To the right are indicated the freezing temperatures and the correlation coefficient for each equation and temperature. Open circles are minimum values for the sampling, 1972-02-05, and solid circles are from 1972-03-21. Degrees of freedom are 18 and 20, respectively.

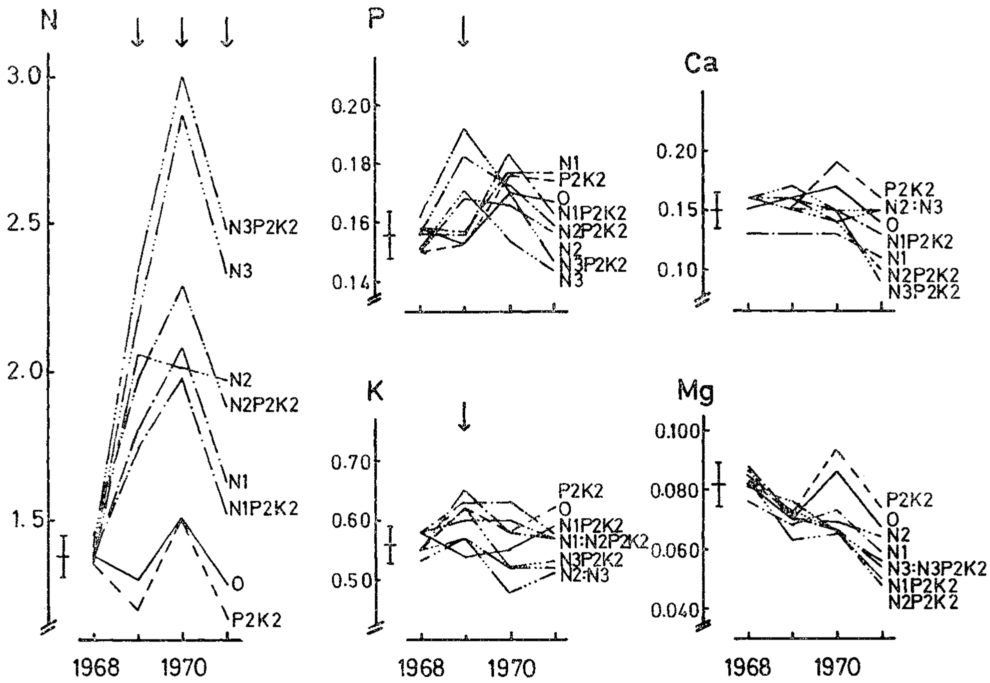



Figure 10. Concentrations of macronutrients in exposed current needles expressed as % dw for many of the plots used in laboratory freezings. Values for the control treatment are the average of five plots, whilst all other values are the average of two plots. In each subfigure, the mean value and standard deviation for all plots are shown for 1968. The scales on the Y-axes are chosen in such a way that standard deviation in each subfigure is about the same length. Fertiliser applications of the nutrient in question are indicated by arrows.

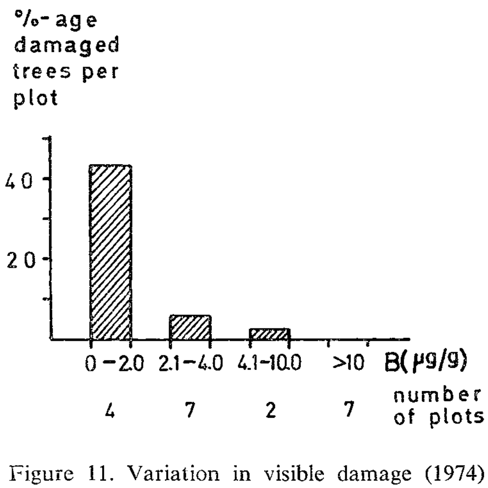



Figure 11. Variation in visible damage (1974) for some plots vis-à-vis the boron content in current needles 1975.

likely that much damage resulted. However, on the nights of the 9th and 10th of June, temperatures were respectively —3.0 and —4.0°C, which might well account for the observed damage (see below).

Moderately low temperatures during the growing season often create so-called frost rings (Dietrichson, 1964; Glerum & Farrar, 1966; Kaiyo et al., 1972). These are caused by low-temperature damage of the cells in the cambial zone. As the cell rows in the xylem of conifers are very regular, it is easy to detect a disturbance. Each of the above two periods with low temperatures resulted in frost rings in the trees. Figures 13 and 14 provide an example of rather slight injury. Only a few, if any, of the cambial cells are destroyed and the bulk of the damaged cells can be assumed to be xylem mother cells and as yet undiffe-

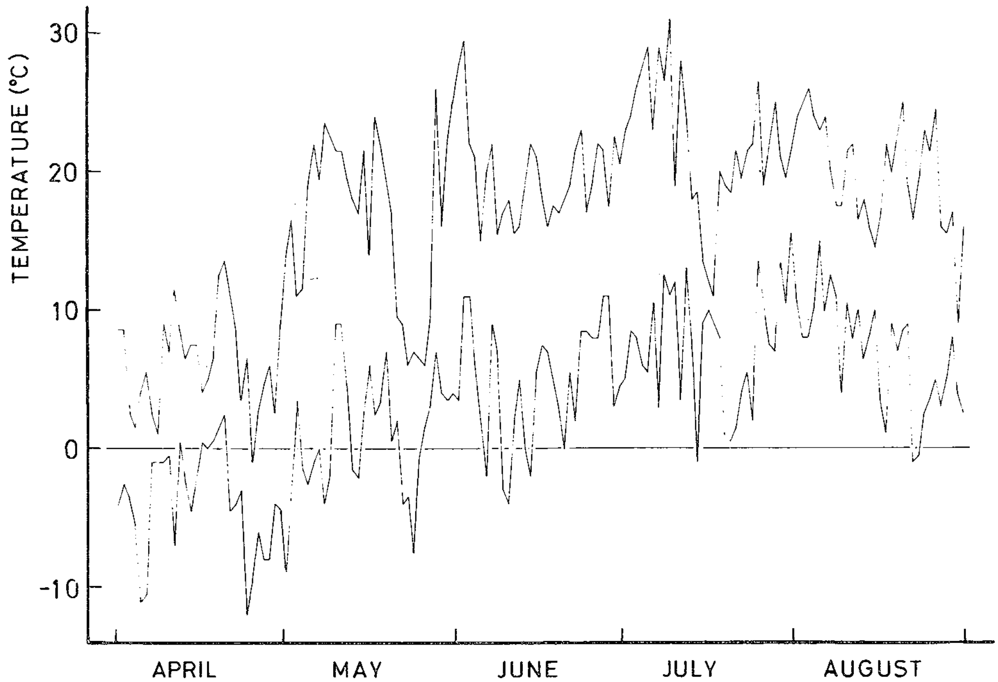



Figure 12. Daily minimum and maximum temperatures during part of 1971, recorded in the temperature cage at the experimental site.

rentiated tracheid cells (Glerum & Farrar, 1966). When the weather improved the rows of xylem cells were completely restored. With serious damage the disturbance is more pronounced and new cells divided from the cambium will not differentiate to normal tracheid cells, but will stay at a parenchyma stage (Glerum & Farrar, 1966). Within these parenchyma cells, the differentiation to xylem cells may occur at a later stage, restoring the normal development of the tissue. However, if the frost is very hard, no cambial cells will survive, the affected part of the shoot will die, and the damage will thus be visible to the naked eye.

The artificial freezing of shoots on standing trees, which was done in June, 1972, resulted in frost rings with damage increasing with a decrease in freezing temperature. The natural frost rings from 1971 and the artificial frost rings in 1972 showed the same characteristics. Branches that were most seriously affected had damage visible to the naked eye and sometimes parts of the shoot had died.

Measurement of tree growth in the three experiments at Lisselbo was performed every three years since the autumn, 1971. An inventory was also made of the visible damage caused by heavy frosts at the beginning of the summer of 1971. The percentage of trees damaged in each plot is plotted against the current needle content of N (Figure 15). At a N-content lower than 2 % dw very few trees were damaged. But at contents higher than 2 % N there were many plots with a high percentage of damaged trees. The K content in the needles had no relationship with the percentage of damaged trees.

During 1971, the radial increment of 10 trees in each plot of experiment E42 was followed weekly by band dendrometer measurements (Redin, 1972). As the first frost occurred soon after the start of the growing season, it is interesting to study in detail the

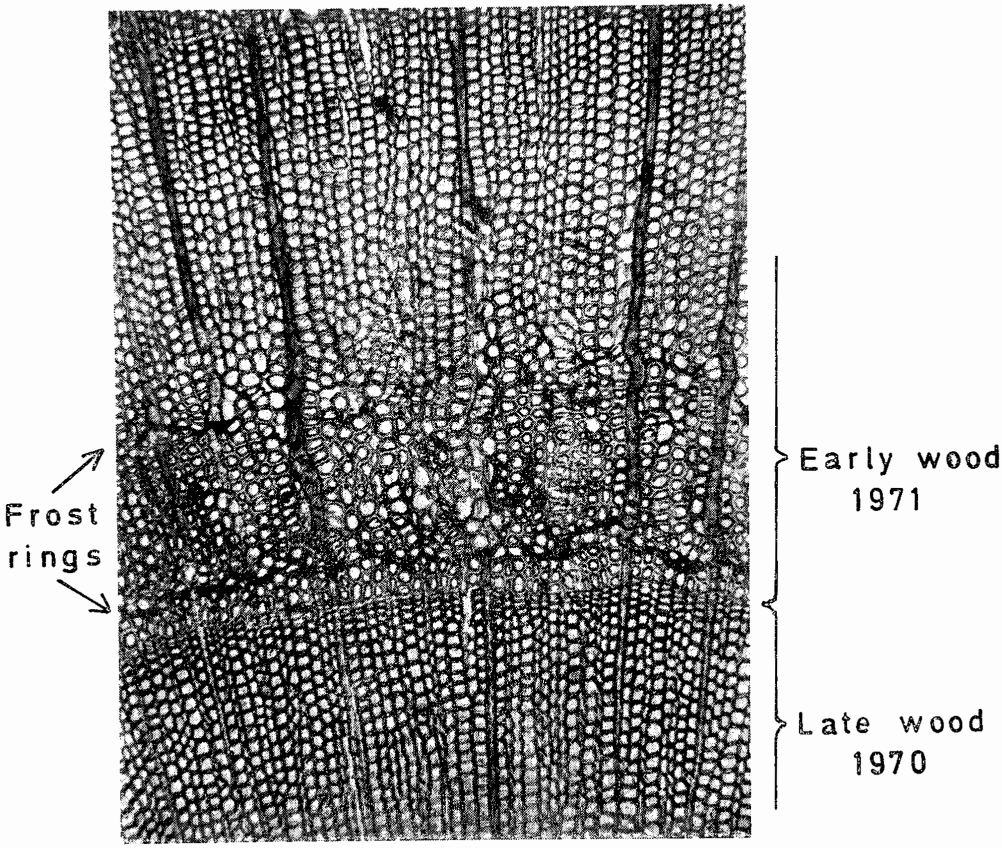
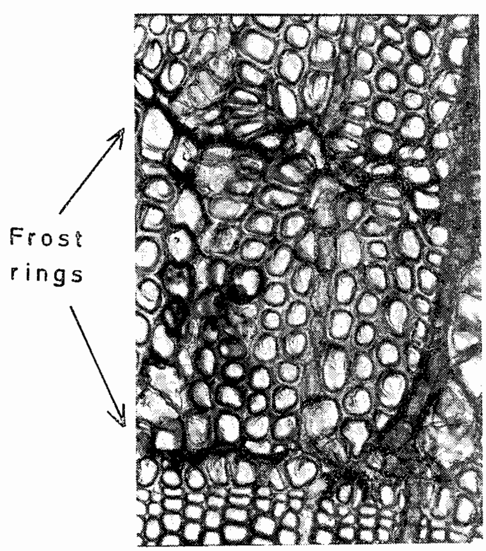



Figure 13. Frost rings caused by the two frost periods in spring, 1971 (May 22 to 24 and June 9 and 10). This particular branch had no damage visible to the naked eye at the sampling occasion in autumn, 1972. 95×.




radial growth immediately before the frost. The girth increment until the 19th of May seems to be closely correlated with the N-content of the needles (Figure 16 A). However, if the growth between the 13th and 19th of May is expressed as percentage of the whole growth for 1971, there is no difference between the plots. That means that the trees with higher nitrogen content were growing faster but all trees were growing at their characteristic rate when the frost occurred. No indication was obtained that N-content affected the stage of growth at this time.

Figure 14. Detail from another part of the same cross section as in Figure 13. 180×.

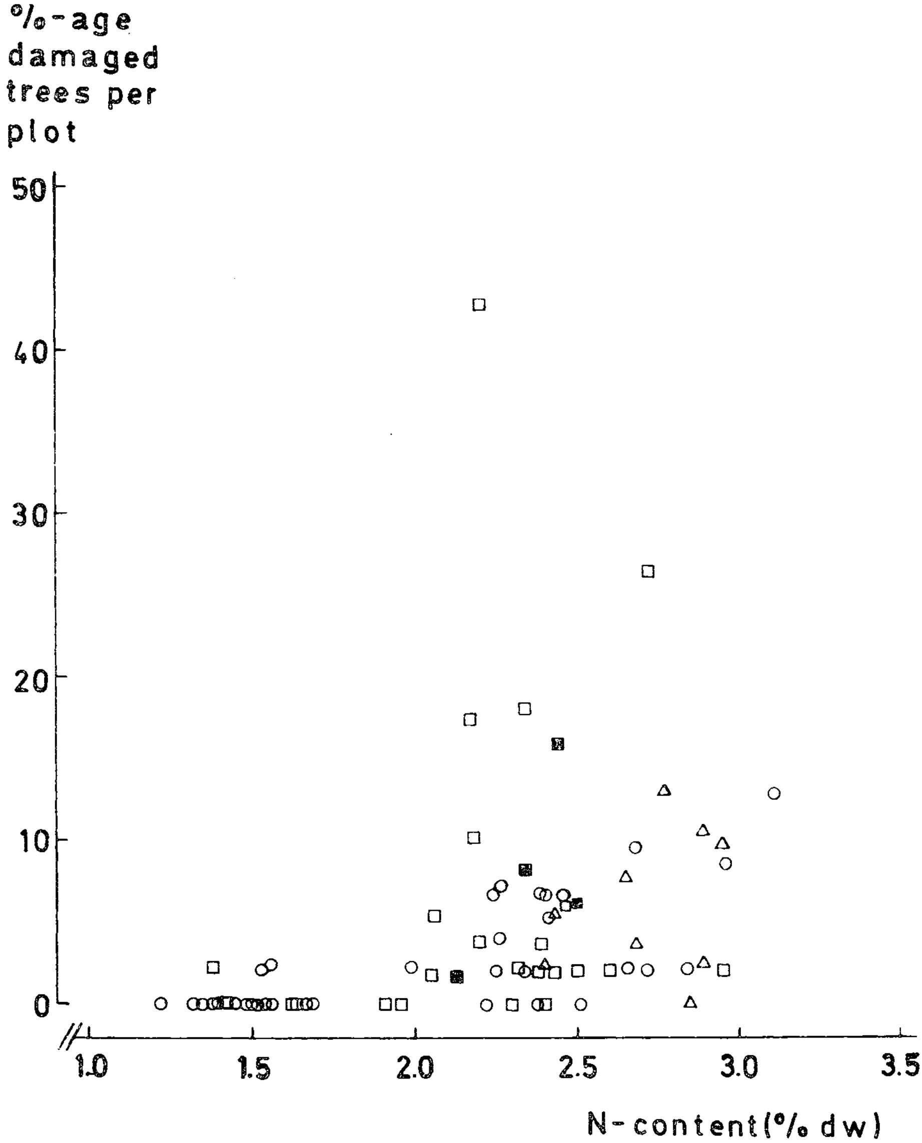



Figure 15. Visible damage very probably caused by the spring frosts of 1971 plotted against the needle contents of nitrogen (% dw). The inventory of damage was done at the regular time for growth measurements in the autumn, 1971, as were the needle samplings for analysis of mineral nutrients. Symbols: △ K-content lower than 0.49 %, ○ K-content between 0.50 and 0.60 %, □ K-content above 0.61 % dw. ■ K-content above 0.61 % and the plots fertilised with a micronutrient mixture (with B, Zn and Cu) in the spring, 1970.

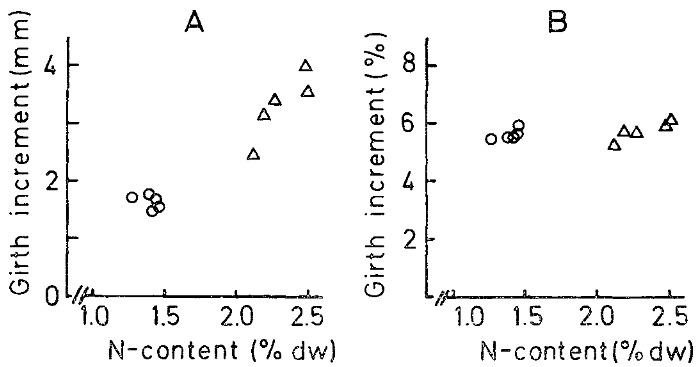



Figure 16. Girth increment of pine trees in E42 measured by band dendrometers at 1.3 m above ground. Usually 12 trees per plot were measured but in a few cases the number of trees was smaller. E42 consists of 20 plots all of which were used. Each symbol is the average of two plots replicate in each treatment. A: Girth increment in mm between the dates 4th to 19th of May. B: Girth increment between the dates 13th to 19th of May expressed as per cent of the total increment for the whole of 1971. Symbols: ○ not fertilised with N2P2K2, but with or without other treatments. △ fertilised with N2P2K2 and with or without other treatments.

# 4 Discussion

In the freezing treatment used here, the cooling rate was very high. For the needles, it can be calculated that they had attained a final temperature (±1.5°C) within 10 minutes of being placed in the deep-freeze (Aronsson & Eliasson, 1970), corresponding to a reduction in temperature of several degrees per minute. In the natural situation, temperature changes will normally occur much more slowly. Levitt (1972) states that a few degrees per hour is normal. However, Sakai (1966) has demonstrated that, in sunshine, rapid fluctuations can occur in a broad-leaved species (*Aucuba Japonica*), and still faster temperature changes have recently been shown for pine needles by Christersson & Sandstedt (1978). If the needles were protected from wind, and the shelter then removed, the temperature dropped more than five degrees per minute. Still faster falls of temperature have been reported for foliage of American arborvitae (*Thuja occidentalis* L.) by White & Weiser (1964). At sunset the temperature could sometimes drop from 35°F to 18°F (about +1.7°C to —7.8°C) within one minute. It can therefore be assumed that during late winter or early spring, the temperature fluctuations during sunny days will be very fast on a transition from sunshine to shadow or vice versa. If then, the air temperature is low enough for the needles in the shade to freeze, several freezings per day may occur (Christersson & Sandstedt, 1978). Langlet (1929), in reporting frost damage to pine in the northern part of Sweden, stated that damage was caused by many fast temperature fluctuations under unusual weather conditions. It is also very probable that frost damage during the winter is often caused by more than one freezing, especially as the plants during that time have very little opportunity to restore damaged cells because of a low rate of metabolic activity (Senser et al., 1975; Senser & Beck, 1977; Palta & Li, 1978).

In the present investigation, repeated freezings of the same needles increased the damage without changing the relative extent of damage between treatments (Figure 6). Although the method used here may differ from a natural freezing incidence, it seems valid to conclude that the differences obtained in RC amongst treatments reflect true differences in frost hardiness.

The relatively big differences in frost hardiness between different trees within the same plot (Figure 5) may to some extent depend on different seed origin, since some of the trees were from seed-trees within the area and others from stands about half a degree north of the experimental area. It is also possible that the differences between plots during the spring frosts (Figure 15) may partly depend on the same factor.

The starting point for the present investigation was the observation that pine shows considerable variation in frost tolerance (Langlet, 1936) and that this variation might well be related to the nutrient status of the trees in addition to other known sources of variation, such as provenance (Eiche, 1966; Kiellander, 1970 a, b; Aronsson, 1977). Suitable material was made available for such an investigation from pine grown at different nutrient regimes in the field plots at Lisselbo, where strong variations in needle N concentrations existed. It was postulated that observed damage to the Lisselbo trees, which at that time was considered frost (or at least winter) damage, might be related to the variations in N concentration.

As described above, a clear correlation has been obtained between frost hardiness and N concentration in pine needles. However, a more definitive interpretation of the experimental results depends on whether other physiological variables, in particular the levels of other mineral nutrients, influence frost hardiness. It is well established that unbalanced nutrient supply affects the nutrient status by e.g. ion antagonism dilution effects (false antagonism). Such effects have also been observed in the present material (Table 2). High nitrogen concentrations led to low concentrations in some other elements and, therefore, the possibility that an induced imbalance in the nutrient status might have affected frost hardiness must be acknowledged.

Potassium has often been reported as having a positive effect on frost hardiness (Levitt, 1956; White & Finn, 1964; Burgtorf, 1968; Alden & Hermann, 1971). However, recent research indicates that K has no great effect on resistance against low temperatures (Benzian et al., 1974; Christersson, 1973, 1975; Larsen, 1976, 1978a). Adequate K concentrations are however recognised as being of great importance in drought resistance (Christersson 1972, 1973, 1976; Larsen, 1976, 1978a, 1978b). In field experiments, water stress often occurs in conjunction with or after low temperatures and, therefore, the importance of K in frost hardiness may have been overestimated. Damage caused by the late spring frost in 1971 (Figure 14) in the present experiment is seen to be unrelated to the K concentrations.

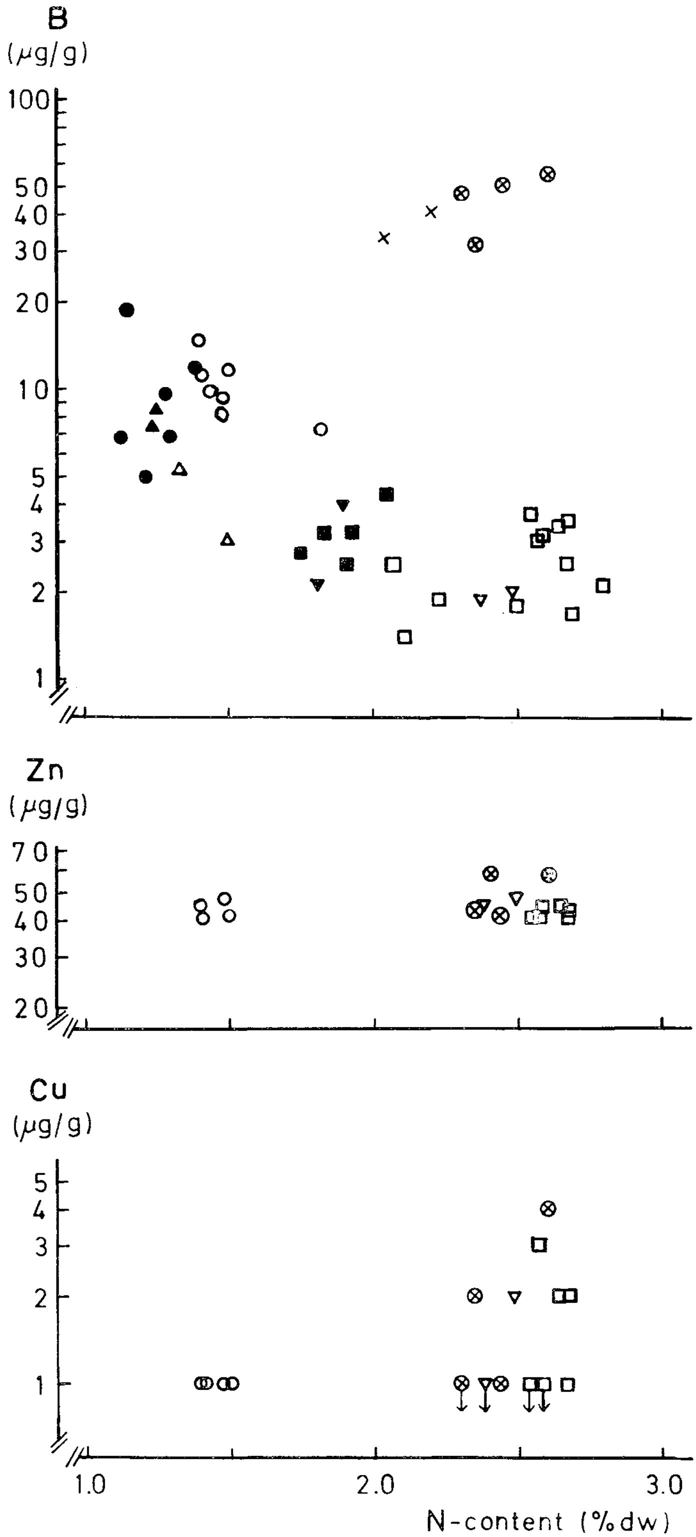
Increase in P concentration is usually considered to give positive effects on frost hardiness (Levitt, 1956; Alden & Hermann, 1971). Malcolm & Freezaillah (1975), however, report that in a fertiliser experiment with seedlings of Sitka spruce with two different P concentrations, seedlings with the higher concentration were more damaged by an early autumn frost than those with lower concentrations. The authors explain the differences in hardiness by the observation that the seedlings with the higher P concentration had not terminated their growth at the time of frost as had the other seedlings. The P concentrations reported were 0.136 and 0.436 per cent dry weight, so that the higher concentration might well be supra-optimal.

There is thus a great variety of opinion on how different plant nutrients affect frost hardening and hardiness. One possible reason is that many investigators have worked with experimental material with rather extreme concentrations of one or more elements. Such studies have much less applicability to ecological conditions than studies where the concentrations of the element in question have been closer to a physiologically balanced condition. Furthermore, many reports lack data on nutrient concentrations and only report fertiliser regimes.

The discussion above may be summarised in the following way: The variation in concentrations of plant nutrient other than N (Figure 10) do not give any support to assumptions that the observed variation in frost hardiness is related to the concentrations of these elements.

However, analyses of needles from different treatments were also made with respect to the micronutrients B, Zn and Cu, because of the damage described below and because of the results from Finnish investigations (Huikari, 1977). As demonstrated in Figure 17, there seems to be a negative relationship between B concentration and N at Lisselbo. This is also the case at two other north Swedish sites, Norrliden and Åheden. In the case of Cu and Zn foliar levels, no effect of nitrogen fertilisation could be observed. In 16 plots at Lisselbo with different nutrient regimes, the variation in Zn concentration ranged from 41 to 58 micrograms per g dry weight and for copper from less than 1 to 4 micrograms per g in the autumn of 1975. The possible relationship between B concentration and frost hardiness is discussed later.

The freezing test in the laboratory gave minimum damage at a N concentration between 1.3 and 1.8 % dw (Figures 7—9). With decreasing test temperature, injuries increased but, in addition, the minimum RC value was displaced in a regular way towards higher N concentrations (Figure 9). The cause of this displacement is not known but it may have something to do with the




Symbols Lisselbo

- ○ Not fertilised
- △ Ca
- ▽ N2P2K2Ca
- □ N2K2 with or without P2MgS
- ⊗ N2K2 micro with or without P2MgS

Symbols Norrliden and Åheden

- ● Not fertilised
- ▲ Ca
- ▼ N2P2K2Ca
- ■ N2P2K2
- × N2P2K2 micro

Figure 17. Contents of B, Zn and Cu vis-à-vis N content in the same needles. Values from different years between 1968 and 1976. The arrows indicate that the content of Cu was 1 $\mu$g/g or lower.

freezing method. Tests at lower temperatures differ not only in absolute temperature but also in the rate of cooling (Aronsson & Eliasson, 1970; Christersson & Krasavtsev, 1972).

The extensive literature on the effect of nitrogen fertilisation on frost hardiness most often reports that increased concentrations in nitrogen have a negative effect but there are also reports of the opposite, as well as conclusions that nitrogen has no effect whatsoever. (For reviews see Levitt, 1956; Alden & Hermann, 1971; cf. also Benzian et al., 1974; Koskela, 1970; Pümpel et al., 1975). However, it is possible that apparent contradictions may be explained by the fact that some authors have studied plant material only within the range of N concentrations below the point where maximum hardiness is obtained, whereas others have worked within a N-sensitive range. Larsen (1978a) reports that in Douglas fir, frost hardiness is decreased by both low and high nitrogen concentrations, with the highest autumn hardiness occurring at 1.3 to 1.4 per cent dry weight. It is also possible that, in much the same way as maximum plant growth can only be reached if the concentrations of mineral nutrients are present in rather fixed proportions (Ingestad 1974), a well balanced plant can endure a stress situation better than a plant with an unbalanced composition of mineral nutrients.

In the present study, the spring frost damage in 1971 also shows that the risk of damage increases at N concentrations above 1.8 to 2.0 per cent (Figure 14). On the other hand, it is not possible to draw conclusions from these data on decreased frost hardiness at low N concentrations. As many plots with high N concentrations are without damage there must be other factors contributing to injury. Low K contents have been thought to be one such factor but some of the worst damaged plots had a relatively high K level (Figure 15). Similar results suggesting that potassium does not affect frost hardiness have been reported by Christersson, 1973, 1975; Larsen, 1976, 1978a; Benzian et al., 1974.

Since 1971—72, when the freezing tests reported here were carried out, it has been observed that injuries seen in the field appear to be more complicated than had at first been assumed. New injuries have also been observed with varying frequencies in the following years. One type of injury consists of abnormal development of the annual shoots in the upper part of the tree, where parts of the shoot or entire shoots die back. The needles may be short and twisted and unevenly developed along the shoots. There are strong indications that such damage is related to deficiency in boron. At the Lisselbo growth survey in 1974, reports from Finnish colleagues were available, indicating that deficiency in boron might be the cause of damage of this type. Therefore, analyses of plant material with respect to boron content were carried out. It is clear from Figure 11 that there is a clear relationship between incidence of damage and boron at low concentration. The concentrations at which damage has been observed are in good agreement with earlier reports for different pine species (see, for example, Snowdon, 1971). Boron concentration was negatively related to the N concentration (Figure 17). Deficiency in boron has been reported to decrease frost hardiness (Cooling, 1967). For this reason, the boron fertilised plots in Figures 7 and 8 have been marked but there does not seem to be any deviation from other plots in these diagrams. This does not of course exclude an effect of B on frost hardiness at low concentrations. This is also in agreement with the results reported by Larsen (1976, 1978a) for Douglas fir where he found no change in frost hardiness by increasing the B concentration by 600 per cent (from 27 to 167 ppm dw). However, there is a relatively narrow range between deficiency and toxicity in the case of B. Deficiency has been reported at concentrations in the range 5—10 ppm in Pinus species and toxicity may sometimes occur at concentrations as low as 75—150 ppm dry weight in needles (Stone, 1968). It is therefore possible that the boron fertilised Douglas firs in Larsen's experiment had supra-optimal B concentrations.

# Summary


Frost hardiness in exposed current needles from young pines in a fertiliser experiment was determined during winter. For the determinations, needles were quickly frozen in deep-freezes at preset test temperatures (—12°, —22°, —32°, and —44°C). By this rapid cooling, it was possible to test the relative frost hardiness of plant material. Damage due to the freezing treatments was determined by conductivity measurements on water extracts of the plant material.

Within the experimental area, there are three different fertiliser experiments, namely, a nitrogen dosage experiment (E40), a factorial experiment (E41), and a mixed experiment with irrigation, acidification or liming (E42). Treatment was begun in the spring, 1969. Nitrogen fertilisation had resulted (1971) in a negative correlation between needle concentration of nitrogen and the other analysed macronutrients (Table 2), possibly attributable in part to increased nitrogen uptake following fertilisation and in part to a dilution effect of other nutrients resulting from the increased growth. Changes of needle concentrations were, however, relatively small for all nutrients except nitrogen (Figure 10) and the variation in frost hardiness found here can be attributed to different nitrogen contents in the needles. Analyses of the mineral nutrients and frost hardiness determinations were made (with a few exceptions) on needles from the same trees.

The freezing experiments during the winters of 1971 and 1972 showed, for relatively heavy damage, a maximum in frost hardiness at a nitrogen concentration of about 1.3—1.8 % dw (Figure 9). At light damage, however, there was no maximum in hardiness and the hardiness decreased somewhat with increasing nitrogen contents of the needles. It is assumed that the maximum in frost hardiness at about 1.5 % N represents that N concentration at which all the mineral nutrients within the needles were in appropriate proportion, not only for frost hardiness, but also for other physiological events.

At the end of May and at the beginning of June, 1971, two relatively hard frosts occurred, which resulted in damage of several trees in many plots. The lightest damage was not visible to the naked eye and occurred only as frost rings in the wood, while at heavy damage, one, two or three year-old shoots died in the upper part of the tree crown. The incidence of damaged trees increased considerably when the concentration in needles was above 1.8—2.0 % N (Figure 15). However, a lot of plots, with high nitrogen concentrations within the needles, did not have any damage, suggesting that a factor or factors other than nitrogen concentration may influence frost hardiness. During the years after 1972 a new type of damage has occurred at Lisselbo. This damage is very probably caused by deficiency in boron and occurs mostly on plots that are fertilised with a high dosage of nitrogen (Figures 11, 17). Therefore it cannot be excluded that deficiency in boron may have had some influence on the damage caused by the spring frosts in 1971. However, fertilisation with boron had not increased the frost hardiness as measured in the laboratory (Figures 7, 8, and 15).

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# Sammanfattning

Frosthärdigheten hos årsbarr från unga tallar från ett av skogsekologiska avdelningens gödslingsförsök (Lisselbo) har bestämts vintertid genom nedfrysningar till några olika temperaturer (—12°, —22°, —32° och —44°C). Med den använda metoden sker nedfrysningen mycket snabbt och även med detta härdiga material erhålles skador vid dessa temperaturer trots att de ligger i ett intervall som förekommer på trädens naturliga ståndorter. Vid utvärderingen av de skador frysningarna på laboratoriet orsakade användes den så kallade exosmosmetoden, dvs., mätning av ledningsförmågan hos vattenextrakt från barren.

Inom försöksområdet Lisselbo finns tre olika slags gödslingsförsök utlagda, nämligen ett kvävedoseringsförsök (E40), ett faktoriellt försök (E41) samt ett försurnings- och kalkningsförsök (E42). Gödslingarna startade 1969. Kvävegödslingen hade 1971 lett till att det förelåg en negativ korrelation i barrhalter för kväve och övriga analyserade mineralämnen (tabell 2). Detta beroende på att gödslingen dels lett till en ökad upptagning av kväve, dels också att detta förhöjda kvävetillstånd gett en större tillväxt med därav följande utspädning av de andra ämnena. Emellertid var haltförändringarna i barren relativt sett små för andra ämnen än kväve (figur 10) och variationen i frosthärdighet kan därför i första hand tillskrivas skillnaderna i barrens kvävehalter. För frysningarna på laboratoriet valdes parceller så att största möjliga spridning i barrens kvävehalter erhölls. Antal provträd per parcell var 10 och dessa var nästan undantagslöst samma träd som de som användes för analyser av barrens halter av mineralnäringsämnen.

Resultaten från frysningarna vintrarna 1971 och 1972 visade att härdigheten vid relativt stora skador avtog vid både låga och höga kvävehalter i barren. Vid kurvanpassning av mätvärdena för skadorna låg den största härdigheten vid en kvävehalt av ca 1.3—1.8 % (figur 9). Vid små skador fanns emellertid inget sådant maximum utan härdigheten minskade svagt med ökande kvävehalt. Orsaken till att härdigheten var som störst vid kvävehalter omkring 1.5 % är troligen att sammansättningen av barrens mineralnäring var bättre balanserade vid dessa halter än vid högre eller lägre halter.

I slutet av maj och början av juni 1971 inträffade två relativt hårda froster. Inom vissa parceller orsakade frosterna i en del fall skador alltifrån frostringar i veden (ej synliga skador) till att en eller flera skottgenerationer i kronans övre delar dog. Frekvensen synligt skadade träd ökade starkt när barrens kvävehalter översteg 1.8—2.0 % av torrvikten (figur 15). Emellertid fanns det många parceller utan några skadade träd trots höga kvävehalter i barren, vilket betyder att fler faktorer än kvävetillståndet inverkade på frosthärdigheten. Bilden kompliceras av att en ny typ av skador har uppträtt inom Lisselbo-försöket på senare år. Dessa skador är troligtvis orsakade av borbrist och förekommer främst på de kraftigast kvävegödslade parcellerna (figur 11 och 17). Det kan därför inte uteslutas att borbrist har bidragit till vårfrostskadorna 1971. Däremot har inte borgödsling gett någon ökning av frosthärdigheten mätt i laboratorieförsöken (figur 7, 8, 15).

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