STUDIA FORESTALIA SUECICA

Nr 103 · 1973

The effect of inorganic nutrients on water economy and hardiness of conifers

I. The effect of varying potassium, calcium, and magnesium levels on water content, transpiration rate, and the initial phase of development of frost hardiness of Pinus silvestris L. seedlings

Samband mellan mineralämnesinnehåll, vattenhushållning och härdighet hos barrträd

I. Effekter av varierande kalium, kalcium- och magnesiumnivåer på vatteninnehåll, transpirationshastighet och frosthärdighetens initialfas hos tallplantor

LARS CHRISTERSSON
Department of Plant Physiology,
University of Lund, Sweden

SKOGSHÖGSKOLAN
ROYAL COLLEGE OF FORESTRY
STOCKHOLM

Abstract

ODC 232.322.41: 174.7 (485) + 161.16 + 181.221.1

The effect of varying amounts of potassium, calcium, and magnesium on water contents, transpiration rates, and frost hardiness of unhardened and 3 weeks' hardened seedlings of Pinus silvestris L. was investigated. The seedlings were 12 and 15 weeks old, respectively.

A hardening period of three weeks (short days: 8 hours light and low temperature: 3°C) decreased the water content of the shoots by 1—6 per cent, and the transpiration rates by about 50 per cent. Increased potassium content increased the water content of the shoot, but decreased the transpiration rate. Increased magnesium content increased the water content, but did not affect transpiration rate. Increased calcium content had no significant effect on water content or transpiration rate.

Different contents of potassium, calcium, and magnesium had no effect, or only a very small effect, on frost hardiness in unhardened or 3 weeks' hardened seedlings.

A transport of potassium from the root to the shoot during hardening was demonstrated and this transport is discussed in connection with winter survival.

Ms. received 20th March 1973

Allmänna Förlaget

ISBN 91-38-01559-5

Berlingska Boktryckeriet, Lund 1973

Contents

Introduction 5

Materials and methods 7
  Plant material 7
  Transpiration measurement 8
  Chemical analysis 8
  Frost hardiness determination 8

Results 10
  The internal ion contents 10
  Growth 10
  Water content 10
  Transpiration rates 11
  Frost hardiness 12

Discussion 18
  Water content 18
  Transpiration rates 18
  Potassium transport 19
  Frost hardiness 20
  Practical applications 21

Acknowledgements 22

Sammanfattning 23

References 25

Introduction

Considerable evidence has accumulated over the years indicating that univalent ions tend to disperse the cell colloids and increase the permeability of the cytoplasm and of the cell membranes, while divalent ions tend to coagulate colloids and decrease the permeability (see e.g. Van Steveninck 1965). A large volume of data on the effect of potassium, calcium, and magnesium on the water economy of higher plants has been collected. On the basis of these data it is considered that an increase of potassium in the cytoplasm of the cell causes swelling of the cytoplasm and a decrease in transpiration rate. On the other hand, an increase in calcium causes shrinkage of the cytoplasm and an increase in transpiration rate. Magnesium is considered to have an intermediate position (Biebl 1958).

Furthermore it is concluded that calcium ions participate in building up and maintaining the membrane systems of the cytoplasm in a functional condition (Burström 1968) and that calcium is essential for the maintenance of the ultrastructure of the cell (Marinos 1962 a). Potassium, on the other hand, participates in the metabolism of the carbohydrates and in protein synthesis (Marschner 1969) as an activator for many enzymes (Mengel 1968). Magnesium is essential to the plant as a part of the chlorophyll molecule and as an activator of various enzymes (Mengel 1968).

Most of these observations derive from experiments with cereals. Very few comparative experiments with conifers have been published. However, Lyr et al. (1967) state that in trees not only potassium and calcium, but also magnesium, influences the colloidal-chemical state of the cytoplasm, especially the degree of swelling.

In silviculture today much effort is directed to finding efficient methods of growing first class seedlings of conifers as cheaply as possible. There have been experiments to find the optimum conditions for rapid growth, e.g. use of plastic houses (Sirén 1968); to find optimum nutrient levels (Ingestad 1970, 1971), water regime (Elowson and Perttu 1970), and carbon dioxide concentration (Aldén 1971). However, some forest tree breeders argue that rapidly grown seedlings have a low capacity to frost and drought hardiness.

The effect of inorganic nutrients on the development of frost hardiness of plants has recently been reviewed by Alden and Hermann (1972). Most work has been with herbaceous plants and only a little with conifers. Besides, investigations of the effect of inorganic nutrients on frost hardiness of conifers have very often been made in field experiments (Benzian et al. 1966, Burgtorf 1968), in which it is difficult to decide whether the death of the seedlings has been caused by cold or drought injuries (Christersson 1972 a).

From field experiments it is known that an increase in potassium content in the leaves increases the survival percentage of seedlings after a severe winter (Benzian et al. 1966, Burgtorf 1968). Calcium has also been reported to increase frost hardiness (see Levitt 1956).

The aim of the present work has been to find out whether or not it is possible to influence the water economy and the development of frost hardiness of pine seedlings by fertilization with varying amounts of potassium, calcium, and magnesium and whether this can be done while maintaining a maximum or almost maximum growth rate. Simple and rapid methods have been used to allow rapid testing of the three ions in concentrations in shoots and in roots that are associated with rapid growth. There were no experiments with slow-growing seedlings suffering from severe deficiency.

The initiative to this investigation was taken by Professor T. Ingestad at the Department of Plant Ecology and Forest Soils, The Royal College of Forestry, Stockholm, Sweden, and throughout the investigation very close co-operation and interesting discussions were established with members of that Department. The present investigation is a part of a wide research project about nutrient requirements, nutrient status, and fertilization effects of forest trees going on at The Royal College of Forestry, Stockholm, Sweden.

Materials and methods

Plant material

The plant material consisted of 12 or 15 weeks old seedlings of Pinus silvestris L. The seeds were from a stand in central Sweden at lat. 57°45'N and altitude 200—300 m (provenance Södra Ydre). They were sown in sterilized gravel in a growth room at 20°C with continuous illumination. They germinated after 5 days and on the fourteenth day the seedlings were mounted on polythene holders. Only seedlings that had shed their seed coats were selected and their was rigorous selection of seedlings of the same appearance for planting, in order to obtain as homogenous material as possible. The seedlings were grown in batches of 10 in 2 l beakers in the basic complete nutrient solution (Table 1) diluted 10 times. The pH varied between 4.5 and 4.0 during the growth period. The nutrient solutions were renewed weekly and the water losses were made good every 2 days with distilled water. The nutrient solutions were well aerated throughout the experiments. Continuous illumination of the growth room was provided by fluorescent tubes, General Electric F48 Power Groove 17-CVX 110 W. The illumination falling on the shoots varied between 10,000 and 13,000 lux, determined with a luxmeter (Gossen Trilux). The total energy supply varied between 25 and 30 $W \cdot m^{-2}$. The temperature of the room was 20.0 ± 1.0°C and the relative humidity 50 ± 10 per cent. There was a continuous flow of air through the room, of 0.2—0.5 $m \cdot s^{-1}$. After four weeks the seedlings were transferred to experimental nutrient solutions.

There were three experimental series in which the concentration of either potassium, calcium, or magnesium in the nutrient solutions was varied. The concentration ranges were chosen so that there were only small differences in growth rates (Ingestad 1962). The variation of the three ions are shown in Table 2: in all the series chloride was the anion. At a low concentration of potassium and magnesium, sodium was used as a cation to keep phosphate or sulphate,

Table 1. Concentration of the elements in the basic nutrient solution. The pH of the basic soltuion was 4.5.

Element	Concentration of the element	Source
N	3.50 $mmol \cdot l^{-1}$	NH_4NO_3
P	0.65 $mmol \cdot l^{-1}$	KH_2PO_4
K	1.25 $mmol \cdot l^{-1}$	KCl, KH_2PO_4
Ca	1.00 $mmol \cdot l^{-1}$	$CaCl_2$
Mg	0.60 $mmol \cdot l^{-1}$	$MgSO_4$
S	0.60 $mmol \cdot l^{-1}$	$MgSO_4$
Fe	23 $\mu mol \cdot l^{-1}$	Fe – EDTA
Mn	3 $\mu mol \cdot l^{-1}$	$MnCl_2 \times 4H_2O$
B	16 $\mu mol \cdot l^{-1}$	H_3BO_3
Zn	0.3 $\mu mol \cdot l^{-1}$	$ZnCl_2$
Cu	0.4 $\mu mol \cdot l^{-1}$	$CuCl_2 \times 2H_2O$
Mo	0.03 $\mu mol \cdot l^{-1}$	$Na_2MoO_2 \times 2H_2O$.

Table 2. The variation of potassium, calcium, and magnesium supply in the experimental series. When one ion was varied the concentrations of the other two were kept constant, as indicated by figures in italic.

Concentration of the varied ions in the nutrient solution, mM		
K	Ca	Mg
0.050	0.003	0.0062
0.125	0.01	0.062
1.25	0.1	*0.62*
3.75	*1.0*	1.9
11.3	3.0	6.2

respectively, at unchanged levels. The concentration of the other ions were the same as in the basic nutrient solution (Table 1). When transferring the seedlings to the experimental nutrient solutions, the two most aberrant seedlings from every beaker were rejected. For every concentration 4 beakers were used, with a total of 32 seedlings. The seedlings were grown for 8 weeks in the experimental nutrient solutions, which were renewed once a week.

After 12 weeks in the nutrient solutions, the pine seedlings stopped their growth in length and developed terminal buds. Half the seedlings were then transferred to another growth room for artificial cold acclimatization. The other half were used for experiments after one or two days. The temperature of the acclimatization room was 3 ± 2°C; the relative humidity was 75 ± 10 per cent; and illumination was as before, but for only 8 hours a day. The seedlings were subjected to this short day and low temperature treatment for 3 weeks before the experiments took place.

Transpiration measurement

After a growing period of 12 weeks for unhardened and 15 weeks for cold-treated seedlings, the transpiration rate of the plant material was determined gravimetrically in the 20°C growth room, illumination 15,000—17,000 lux, airflow 1 $m \cdot s^{-1}$. Four seedlings were investigated from each concentration of each of the three ions. These seedlings were grown in the same nutrient solution as before. Before measurement the cold-treated seedlings were kept in the 20°C room for 12 hours, during which time the temperature of the solutions reached 20°C.

After the transpiration experiments the fresh and dry weights were determined, the latter after drying overnight at 105°C.

Chemical analysis

The shoots and the roots of the seedlings used in the transpiration experiments were separated and the potassium and calcium contents were measured after ashing at 450°C with an Eppendorf flame photometer, the phosphorus content was measured colorimetrically (Beckman spectrophotometer, model C) (Youngberg and Youngberg 1930) and the magnesium content was measured with an Aminco-Bowman spectrophotofluorometer (Olsen and Diehl 1963).

Frost hardiness determination

The remaining plant material was used in a freezing test. The equipment for this test was described in detail by Christersson (1971). The roots of the seedlings were wrapped in wet blotting-paper immediately after they were taken out of the nutrient solutions. The shoot was placed in a test tube and this tube was placed in a bath of 95 per cent ethyl alcohol. The seedlings were upside down during the test. With the aid of a Colora Immersion Cooler TK 67 and four synchronous motors, the temperature of the bath was decreased at a cooling rate of 4°C per hour to the desired temperature. This temperature was maintained for two hours (Christersson and Krasavtzev 1972) and then increased at a rate of 4°C per hour to +3°C. The course of the freezing test was regulated automatically. The root temperature was 20°C throughout the test.

The temperature at the plant surface was measured by fixing a small silicon diode all along the stem of the seedling near the terminal bud with a string in such a way that the needles of the seedlings completely surrounded the diode. The diode was connected to a potentiometric recorder (Servogor, type 511, Wien) by an electronic circuit.

A typical freezing-test curve is shown in Figure 1. The temperature of the bath was +3°C at the beginning of each test. The lowest temperature was between −4.0°C and −6.7°C in tests of unhardened seedlings and between −6.1°C and −13.0°C in tests of hardened seedlings. A small piece of ice was transferred to the test tube together with the shoot to inoculate ice crystals in the tissue in an early state of the test in order to minimize super-cooling

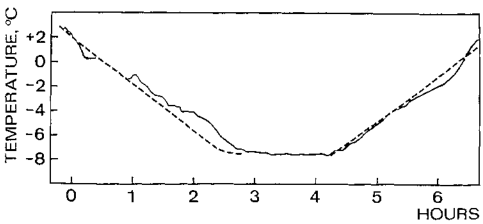

Figure 1. An example of a freezing-test curve. The freezing and thawing rate was 4°C per hour. The duration of exposure to the lowest temperature was 2 hours. Broken line: theoretical test temperature; continuous line: actual test temperature, measured by a small diode, surrounded by layers of needles close to the terminal bud of the shoot.

(Levitt 1956). It was not possible to decide the exact test temperature in advance, as the intended temperature varied within half a degree. The accuracy of temperature measurement was ± 0.3°C.

Five different test-tubes were immersed in the bath at the same time with one seedling in each tube from every concentration of the investigated ion. When a temperature of + 3°C had been attained at the end of the test, the seedlings were transferred back to a complete nutrient solution and kept at + 3°C and 8 hours illumination for 2 days. They were then transferred to darkness and 20°C for 24 hours and then placed in a growth room with continuous illumination at 20°C. After further six days, injuries appearing were assessed and the degree of damage was described in the following way:

+ No sign of injuries at all, or the top of some needles brown, the xylem white.

− 50 per cent or more of the needles brown or dropped off, the xylem brown.

No tested seedlings had injuries to the needles which could be estimated as intermediate between + and −. The colour of the xylem was decided after scratching the stem of the seedling just below the terminal bud with a nail.

In pilot experiments to establish a suitable freezing-test, it was established that seedlings assessed as + were still alive and growing after 2 months, while all the seedlings assessed as − were completely dead after 2 months.

The experiments were repeated once, and the values of the transpiration rates and the water contents were tested statistically by regression analysis, performed by the Statistical Service of the Swedish Natural Science Research Council.

Results

The internal ion contents

The analytical results show that the internal variation of the potassium, calcium, and magnesium contents in the shoots are 0.095—0.48, 0.015—0.14, and 0.012—0.13 mmol per g dry matter, respectively (Table 3, 4, and 5). According to Ingestad (1962, page 118), the maximum growth rates for pine seedlings in water culture fall within these concentration ranges.

Slight deficiency symptoms were developed in the potassium and magnesium series, but not in the calcium series. The tips of the needles were yellow, with the yellow zone much more distinctly separated from the green part of the needle in the magnesium series than in the potassium series. There was no detectable visual effect of excess of the varied elements.

As only concentrations which allowed maximum or almost maximum growth were used, antagonism between ions was not very pronounced. However, at increased calcium supply, the magnesium content of both roots and shoots decreased markedly (Table 4). Increased magnesium supply to the root also decreased the calcium content (Table 5). The variation in the analytical results was usually less than ± 15 per cent of the mean value.

The technique used gave an opportunity to study the influence of a short hardening period on the retention and transport of the ions in roots and shoots. The results demonstrate that the cold acclimatization period had a distinct effect on the potassium distribution. The potassium content of hardened shoots was always higher than that of unhardened shoots, while the potassium content of the root was lower (Table 3, 4, and 5).

Growth

The fresh matter production in the three series (Table 6, 7, and 8) showed the same response to different concentrations of the investigated ions as was demonstrated by Ingestad (1960, 1962) but the growth rates were considerably higher. The fresh weights indicate that the concentrations of the tested nutrient elements produced maximum or almost maximum growth rates under the prevailing conditions. There was a more pronounced maximum for shoot growth in the magnesium series than in the potassium series, while the maximum for the calcium series was flatter. A maximum was established for root growth in the magnesium series, but not in the potassium and calcium series. This means that the fresh weight shoot/root ratio showed a maximum in the potassium series, but not in the calcium and magnesium series. In pilot experiments it was shown that growth stopped completely during the hardening period. The variation in the plant material on the fresh weight basis rarely exceeded ± 25 per cent of the mean value.

Water content

In all series the hardening period of three weeks decreased the water contents of the shoots. This decrease varied between 1 and 6 per cent, and was least pronounced at low levels of potassium and magnesium. The water content of the shoot increased with increasing potassium content (Table 6) in unhardened seedlings ($P < 0.001$). In seedlings hardened for three weeks, the effect of different potassium levels was smaller ($P < 0.05$). Different calcium contents in the shoot (Table 7) do not have any significant effect on the water content

Table 3. The effect of potassium supply on the contents in mmol per g dry matter of potassium, calcium, magnesium, and phosphorus in shoots and roots of 12 weeks' old unhardened and 15 weeks' old hardened seedlings of Pinus silvestris L. Each figure represents a mean value of four determinations on different seedlings. The experiment was repeated once and the results of both the experiments are included in the table.

Potassium supply, mM	Contents, mmol per g dry matter							
	K		Ca		Mg		P	
	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened
	Shoot							
0.050	0.095	0.11	0.050	0.062	0.079	0.062	0.074	0.081
	0.11	0.12	0.045	0.063	0.074	0.072	0.063	0.074
0.125	0.13	0.17	0.043	0.060	0.063	0.070	0.065	0.090
	0.17	0.17	0.065	0.050	0.075	0.064	0.062	0.058
1.25	0.20	0.24	0.058	0.068	0.071	0.070	0.071	0.087
	0.20	0.24	0.077	0.070	0.078	0.069	0.071	0.077
3.75	0.22	0.25	0.060	0.050	0.075	0.061	0.074	0.058
	0.22	0.24	0.060	0.050	0.063	0.058	0.079	0.058
11.3	0.42	0.47	0.062	0.075	0.069	0.053	0.081	0.071
	0.41	0.48	0.063	0.055	0.063	0.052	0.078	0.065
	Root							
0.050	0.11	0.11	0.060	0.050	0.063	0.069	0.13	0.12
	0.10	0.11	0.058	0.047	0.069	0.063	0.11	0.14
0.125	0.19	0.14	0.055	0.050	0.067	0.075	0.12	0.13
	0.25	0.19	0.048	0.050	0.061	0.067	0.12	0.12
1.25	0.30	0.27	0.047	0.060	0.067	0.063	0.13	0.13
	0.30	0.29	0.040	0.045	0.067	0.057	0.12	0.11
3.75	0.42	0.26	0.065	0.048	0.059	0.065	0.16	0.12
	0.42	0.31	0.040	0.043	0.056	0.054	0.10	0.10
11.3	0.45	0.37	0.043	0.040	0.045	0.041	0.10	0.12
	0.51	0.41	0.050	0.037	0.047	0.047	0.11	0.13

in the shoot whether hardened or not. Results similar to those for potassium were obtained for magnesium (Table 8) in unhardened seedlings ($P < 0.001$); in hardened seedlings magnesium content have no significant effect on water content.

Transpiration rates

A hardening period of three weeks had a definite effect on the transpiration rates: the transpiration rates of hardened seedlings were about half those of unhardened seedlings, for all the experimental series (Table 6, 7, and 8). However, no correlations between transpiration rates and water contents or shoot/root ratios could be established. The three ions had different effects on transpiration. The transpiration rate decreased at very high potassium contents in unhardened seedlings ($P < 0.01$) (Table 6). In hardened seedlings there was no significant effect of increasing potassium contents in the shoot on the transpiration rate. The transpiration rates showed a maximum at increasing calcium content for hardened seedlings ($P < 0.05$) (Table 7), but the effect on unhardened seedlings was not significant. There were no significant effects of different magnesium contents on the transpiration rates (Table 8).

Table 4. The effect of calcium supply on the contents in mmol per g dry matter of potassium, calcium, magnesium, and phosphorus in shoots and roots of 12 weeks' old unhardened and 15 weeks' old hardened seedlings of Pinus silvestris L. For further explanation, see Table 3.

Calcium supply, mM	Contents, mmol per g dry matter							
	K		Ca		Mg		P	
	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened
	Shoot							
0.003	0.23	0.29	0.020	0.019	0.086	0.095	0.060	0.061
	0.23	0.29	0.015	0.015	0.078	0.090	0.070	0.061
0.01	0.27	0.38	0.025	0.021	0.086	0.082	0.074	0.051
	0.20	0.38	0.018	0.020	0.071	0.10	0.054	0.073
0.1	0.23	0.34	0.060	0.055	0.091	0.099	0.064	0.075
	0.21	0.38	0.060	0.055	0.070	0.095	0.082	0.068
1.0	0.29	0.31	0.090	0.095	0.078	0.091	0.065	0.055
	0.23	0.33	0.070	0.10	0.070	0.096	0.055	0.075
3.0	0.28	0.31	0.10	0.14	0.062	0.070	0.074	0.058
	0.23	0.38	0.12	0.14	0.062	0.086	0.073	0.081
	Root							
0.003	0.37	0.20	0.015	0.018	0.091	0.095	0.12	0.12
	0.36	0.21			0.095	0.082	0.11	0.12
0.01	0.44	0.28	0.016	0.022	0.066	0.085	0.12	0.11
	0.36	0.23	0.013	0.013	0.074	0.078	0.10	0.14
0.1	0.45	0.30	0.028	0.030	0.070	0.070	0.12	0.10
	0.38	0.26	0.020	0.018	0.062	0.078	0.16	0.12
1.0	0.42	0.29	0.053	0.073	0.054	0.062	0.10	0.11
	0.37	0.23	0.052	0.063	0.051	0.054	0.11	0.11
3.0	0.39	0.35	0.067	0.085	0.038	0.041	0.14	0.11
	0.34	0.22	0.060	0.083	0.045	0.045	0.14	0.12

Frost hardiness

The effect of the investigated ions on the frost hardiness of unhardened seedlings was very small (Table 9). Perhaps very high levels of potassium and calcium led to decreased frost hardiness. Magnesium had no effect on the frost hardiness of unhardened seedlings. An acclimatization period of 3 weeks lowered the survival temperature by 3—5 degrees, but the results show that the initial phase of development of frost hardiness was not affected by these ions (Table 10).

Table 5. The effect of magnesium supply on the contents in mmol per g dry matter of potassium, calcium, magnesium, and phosphorus in shoots and roots of 12 weeks' old unhardened and 15 weeks' old hardened seedlings of Pinus silvestris L. For further explanation, see Table 3.

Magnesium supply, mM	Contents, mmol per g dry matter							
	K		Ca		Mg		P	
	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened
	Shoot							
0.0062	0.20	0.24	0.088	0.078	0.017	0.014	0.055	0.071
	0.26	0.26	0.085	0.070	0.019	0.012	0.065	0.058
0.062	0.21	0.29	0.070	0.078	0.034	0.029	0.074	0.074
	0.23	0.23	0.055	0.052	0.033	0.029	0.065	0.058
0.62	0.20	0.24	0.050	0.053	0.058	0.053	0.081	0.071
	0.25	0.25	0.060	0.060	0.060	0.049	0.090	0.077
1.9	0.25	0.26	0.053	0.075	0.078	0.095	0.084	0.077
	0.27	0.28	0.050	0.078	0.074	0.10	0.096	0.062
6.2	0.27	0.28	0.057	0.050	0.13	0.13	0.068	0.058
	0.25	0.26	0.050	0.060	0.11	0.13	0.082	0.062
	Root							
0.0062	0.26	0.19	0.065	0.070	0.017	0.011	0.11	0.087
	0.32	0.24	0.065	0.055	0.016	0.012	0.090	0.087
0.062	0.29	0.20	0.072	0.070	0.031	0.031	0.10	0.11
	0.30	0.26	0.057	0.063	0.026	0.026	0.090	0.084
0.62	0.29	0.20	0.048	0.048	0.053	0.045	0.12	0.10
	0.39	0.20	0.043	0.030	0.059	0.065	0.097	0.087
1.9	0.32	0.29	0.037	0.040	0.086	0.062	0.13	0.10
	0.36	0.28	0.038	0.033	0.074	0.069	0.12	0.10
6.2	0.34	0.24	0.030	0.028	0.14	0.091	0.15	0.097
	0.36	0.25	0.025	0.027	0.11	0.11	0.14	0.10

Table 6. The effect of potassium supply on the fresh weights of shoots and roots, the shoot/root ratio, the water content, and the transpiration rate of 12 weeks' old unhardened and 15 weeks' old hardened seedlings of Pinus silvestris L. Each figure represents a mean value of four determinations on different seedlings. The experiment was repeated once and the results of both experiments are included in the table.

Potassium supply, mM	Fresh weight shoot, g		Fresh weight root, g		Fresh weight ratio shoot/root		Water content of the shoot, per cent		Transpiration per g dry matter of needles and hour, $mg \cdot g^{-1} \cdot h^{-1}$	
	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened
0.050	2.34	2.00	0.93	0.79	2.52	2.53	73.4	72.3	1260	820
	2.32	1.75	0.96	0.64	2.42	2.73	74.1	71.5	1360	890
0.125	2.64	2.76	0.82	0.80	3.22	3.45	74.4	72.5	1300	840
	2.57	1.97	0.80	0.68	3.21	2.89	75.6	72.5	1450	710
1.25	2.66	2.39	0.92	0.87	2.89	2.75	75.0	74.0	1250	720
	2.77	1.93	1.21	0.72	2.29	2.68	74.7	71.6	1430	1180
3.75	2.96	2.19	0.97	0.84	3.06	2.61	76.1	72.9	1270	710
	2.71	2.15	1.13	0.74	2.40	2.90	75.7	72.7	1420	710
11.3	2.48	2.15	0.89	0.88	2.79	2.45	77.8	73.5	1110	520
	2.48	2.22	1.07	1.16	2.32	1.91	77.8	73.1	1290	600

Table 7. The effect of calcium supply on the fresh weight of shoots and roots, the shoot/root ratio, the water content, and the transpiration rate of 12 weeks' old unhardened and 15 weeks' old hardened seedlings of Pinus silvestris L. For further explanation, see Table 6.

Calcium supply, mM	Fresh weight shoot, g		Fresh weight root, g		Fresh weight ratio shoot/root		Water content of the shoot, per cent		Transpiration per g dry matter of needles and hour, $mg \cdot g^{-1} \cdot h^{-1}$	
	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened
0.003	2.76	2.29	1.14	1.11	2.42	2.06	76.9	72.9	1400	700
	2.13	2.60	0.85	1.32	2.51	1.96	76.8	73.3	1430	690
0.01	2.73	2.02	1.38	0.86	1.98	2.35	76.5	72.8	1560	830
	2.13	2.48	0.86	1.08	2.48	2.30	75.3	73.1	1420	700
0.1	2.47	2.40	1.27	1.18	1.89	2.03	76.0	72.3	1570	930
	2.04	2.29	0.90	1.28	2.26	1.78	77.1	73.8	1690	770
1.0	2.08	2.40	0.93	1.41	2.24	1.70	77.5	73.2	1540	760
	2.00	2.15	1.01	1.02	1.98	2.12	76.3	72.6	1730	730
3.0	2.61	2.14	1.18	0.77	2.21	2.76	76.9	72.9	1420	770
	2.31	2.28	0.87	1.24	2.66	1.83	78.1	72.5	1690	580

Table 8. The effect of magnesium supply on the fresh weight of shoots and roots, the shoot/root ratio, the water content, and the transpiration rate of 12 weeks' old unhardened and 15 weeks' old hardened seedlings of Pinus silvestris L. For further explanation, see Table 6.

Magnesium supply, mM	Fresh weight						Water content of the shoot, per cent		Transpiration per g dry matter of needles and hour, mg · g^{-1} · h^{-1}	
	shoot, g		root, g		ratio shoot/root					
	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened	unhardened	hardened
0.0062	1.68	1.99	0.85	0.94	1.98	2.12	75.8	73.9	1430	730
	1.67	1.58	0.84	1.07	1.99	1.47	75.8	73.9	1130	620
0.062	1.97	1.81	1.16	0.69	1.70	2.62	76.6	74.8	1670	970
	1.65	1.77	1.02	1.14	1.62	1.55	76.9	74.8	1670	790
0.62	2.12	2.25	1.31	1.00	1.62	2.25	76.9	73.7	1470	830
	1.65	1.76	0.86	1.30	1.92	1.92	76.3	73.9	1390	850
1.9	2.63	2.06	1.49	0.93	1.77	2.21	77.8	74.2	1280	720
	1.96	2.21	1.25	1.40	1.56	1.58	77.2	73.6	1180	720
6.2	2.33	1.97	1.23	0.84	1.90	2.35	77.8	74.9	1190	690
	1.54	1.72	0.74	1.00	2.08	1.72	77.8	74.7	1110	590

Table 9. Determination of frost hardiness of 12 weeks' old, unhardened seedlings of Pinus silvestris L. with different contents of potassium, calcium, and magnesium in the shoots. Key to the signs:

+ no sign of injuries or the top of the needles brown, the xylem white.

− 50 per cent or more of the needles brown or dropped off, the xylem brown.

The test results from two different experiments are put together in groups as indicated. Each sign stands for one seedling. Each analytical value represents a mean value of 8 determinations on different seedlings.

Test temperature, °C	Potassium contents in the shoot, mmol per g dry matter				
	0.10	0.15	0.20	0.22	0.42
−4.0 −4.3	+ +	+ +	+ +	+ +	+ +
−4.4 −4.7	+ −	+ −	+ −	+ −	+ −
−4.8 −5.1	+ +	+ +	+ +	+ +	+ +
	+ −	+ −	− −	− −	− −
−5.2 −5.5	+ −	− +	− +	− −	− −
	− −	− −	− −	− −	− −
−5.6 −5.9	+ − −	+ − −	− − −	− − −	− − −
	− −	− −	− −	− −	− −
−6.0 −6.3	+	−	−	−	−
−6.4 −6.7	− −	− −	− −	− −	− −
	−	−	−	−	−

	Calcium contents in the shoot, mmol per g dry matter				
	0.018	0.022	0.060	0.080	0.11
−4.0 −4.3	+ +	+ +	+ +	+ +	+ +
	+	+	+	+	+
−4.4 −4.7	+ −	+ +	+ +	+ −	+ −
	+ −	+ −	+ −	+ +	− +
−4.8 −5.1	+ −	+ −	+ −	+ −	+ −
	+ −	− −	− −	− −	− −
−5.2 −5.5	+ −	+ +	+ −	+ +	− −
	− −	− −	− −	− −	− −
−5.6 −5.9	+ −	+ −	− −	− −	− −
	−	−	−	−	−
−6.0 −6.3	− −	− −	− −	− −	− −
	−	−	−	−	−
−6.4 −6.7	− −	− −	− −	− −	− −
	−	−	−	−	−

	Magnesium contents in the shoot, mmol per g dry matter				
	0.018	0.034	0.059	0.076	0.12
−4.0 −4.3	+ +	+ +	+ +	+ +	+ +
−4.4 −4.7	+	+	+	+	+
−4.8 −5.1	+ −	− −	+ −	+ +	− +
	−	−	−	+	−
−5.2 −5.5	+ + −	+ − −	+ + −	+ + −	+ + −
	− − −	− − −	− − −	− − −	− − −
−5.6 −5.9	− −	− −	+ −	− −	− −
	−	−	−	−	−
−6.0 −6.3	− −	− −	− −	+ −	− −
−6.4 −6.7	− −	− −	− −	− −	− −
	−	−	−	−	−

Table 10. Determination of frost hardiness of 15 weeks' old, 3 weeks' hardened seedlings of Pinus silvestris L. with different contents of potassium, calcium, and magnesium in the shoots. For further explanations, see Table 9.

Test temperature, °C		Potassium contents in the shoot, mmol per g dry matter				
		0.12	0.17	0.24	0.25	0.48
−6.1	−7.0	+ +	+ +	+ +	+ +	+ +
−7.1	−8.0	− −	− −	+ −	+ −	− +
−8.1	−9.0	+ +	+ +	+ +	+ +	+ −
		− −	− −	− −	− −	− −
−9.1	−10.0	+ +	+ +	+ +	+ +	− −
		− −	+ −	+ +	+ +	+ −
−10.1	−11.0	+ +	+ +	+ +	+ +	+ −
		− −	+ +	+ +	+ +	− −
−11.1	−12.0	+ +	+ −	+ −	+ +	+ −
		−	−	−	−	−
−12.1	−13.0	− −	− −	− −	− −	− −
		Calcium contents in the shoot, mmol per g dry matter				
		0.017	0.021	0.055	0.098	0.14
−6.1	−7.0	+ +	+ +	+ +	+ +	+ +
−7.1	−8.0	+ −	+ −	− +	+ −	+ −
		−	+	+	+	−
−8.1	−9.0	+ −	+ −	+ −	− −	− −
		− −	− −	− −	− −	− −
−9.1	−10.0	+ −	+ +	+ +	+ +	+ +
		−	−	−	−	−
−10.1	−11.0	+ +	+ −	+ +	− +	− −
		− −	+ −	+ −	− −	− −
−11.1	−12.0	+ −	− −	− −	− −	− +
−12.1	−13.0	− −	− −	− −	− −	− −
		Magnesium contents in the shoot, mmol per g dry matter				
		0.013	0.029	0.051	0.098	0.13
−6.1	−7.0	+	+	+	+	+
−7.1	−8.0	+ +	+ +	+ +	+ +	+ +
−8.1	−9.0	+ +	+ +	+ +	+ +	+ −
		−	+	+	+	+
−9.1	−10.0	+ − +	− − +	− + +	− + −	− + +
		− −	+ −	+ +	− −	− −
−10.1	−11.0	+ +	+ +	+ −	+ −	− −
		−	+	−	−	−
−11.1	−12.0	− −	− −	+ −	− −	− −
		−	−	+	−	−
−12.1	−13.0	− −	− −	− −	− −	− −
		−	−	−	−	−

Discussion

The rate of fresh matter production and the analytical results indicate that the plant material was cultivated in optimum or nearly optimum concentrations, with maximum or nearly maximum growth rates during the growing period. However, comparing the growth rates and the analytical results with those of Ingestad (1962) and Tamm (1964), it is apparent that in spite of the fact that the variation in size of the seedlings was rather small, the growth rate of unhardened seedlings at the time of the experiment might vary more than the figures of the fresh matter production indicated. This fact ought to be kept in mind in the interpretation of the results.

Water content

In this material, as short an acclimatization period as three weeks decreased the water contents of shoots of Scots pine seedlings. These results agree very well with Levitt (1956), who reviewed the relation between the hardening processes and water contents.

In the present work and in the works cited above the water contents have been expressed as per cent of fresh weight, which consist of water and dry matter. So a decrease in water content as per cent of fresh weight means either a real decrease in water content or an increase in dry matter. Langlet (1936) preferred to call the change in Scots pine needles an increase in content of dry matter. He expressed the dry matter as a percentage of a water deficit corrected fresh weight. Kozlowski and Clausen (1965) and Little (1970), on the other hand, expressed the moisture contents of Pinus strobus, Pinus resinosa, and Abies balsamea as percentages of oven-dry weight. In general, it can be supposed that there are changes both in water content and in dry matter during a hardening period and it is necessary to take the unit of measurement into consideration in interpreting the results. In the present investigation it is not possible to distinguish between changes in water content and changes in dry matter, and it can only be supposed that there are changes in both.

During an acclimatization period the plant material is exposed to low temperature. It is reasonable to believe that a low temperature decreases the water transport from the soil, or in this case from the nutrient solution, to the needles, e.g. by increasing the viscosity of water and of protoplasm and by decreasing root growth and water permeability of the cell membrane (Kramer 1942). This in itself could cause the observed increase in water deficit. In the present investigation this effect of low temperature on the water deficit and consequently on water content is compensated by keeping the plant material at a temperature of 20°C for 24 hours before measuring water content. It seems, therefore, that the decerase in water content of shoots of Scots pine seedlings, expressed as a percentage of fresh weight, depends upon physiological processes going on during a hardening period, and not on decreased water uptake. However, it can be supposed that during a hardening period plants have a large water deficit in the tissues, and possibly this is one of the factors necessary for stopping growth and inducing the beginning of the physiological hardening processes.

Transpiration rates

No correlations between water content and transpiration rate have been demonstrated in the present investigation (Table 6, 7, and 8). However, different levels of potassium or calcium, and the hardening period, had a marked effect on the transpiration rate. High contents of potassium in the needles decreased the transpiration rate. Similar results were obtained for spruce by Keller (1967) and Zech et al. (1969). For rape (Arland and Enzmann 1956), lucern (Blanchet et al. 1962) and wheat (Brag 1972), a decrease in the transpiration rates has also been associated with increasing potassium contents in the leaves. The way in which the change in potassium content influences the transpiration stream is a very intriguing problem. Transpiration rate is to a very large extent controlled by stomatal movements (Stålfelt 1956, page 368) and considerable evidence has accumulated recently that potassium ions are pumped in and out of the guard cells during stomatal opening and closing (Fujino 1967, Fisher 1968, Sawhney and Zetlich 1969, Willmer and Mansfield 1970, Humble and Raschke 1971, Raschke and Fellows 1971, Turner 1972). In the present investigation, the supply of potassium was never very low and, furthermore, the transpiration rates were affected only at high and very high concentrations of potassium, when it can be supposed that the potassium concentration as such cannot be a limiting factor for the stomatal movements. It would be expected that only very low potassium contents in the leaves affect the pumping of potassium in and out of the guard cells.

The transpiration rates are not affected, or are affected very little, by increasing calcium and magnesium contents in the shoot. There are very few comparable results for conifers, but H. Brag (personal communication) has shown that the transpiration rate of wheat reaches a maximum as magnesium content increases.

Parker (1949) assumed a positive correlation between the ratio of root to shoot surface areas and the transpiration rates. In a study of the nutrition of pine seedlings, Ingestad (1960) showed that in concentration series similar to those used in the present investigation the effect of different concentrations of potassium, calcium, and magnesium on the ratio of fresh weights of roots to shoots was very small. In the present investigation a maximum root to shoot ratio was shown for increasing potassium concentrations, but changes in concentration of calcium and magnesium had no effect (Table 6, 7, and 8), so the changes in the transpiration rates cannot be explained by changes in the root to shoot ratio.

After a hardening period of three weeks the transpiration rates decreased by about 50 per cent. Pine and spruce seedlings grown in pots also had reduced transpiration rates after a hardening period of three months (Christersson 1972 a). It was assumed that the stomata of hardened seedlings remain closed even in light at a temperature of 20°C as long as the seedlings were not dehardened. Unfortunately it has not been possible to measure the width of the stomata apertures in conifer needles. Cristersson (1972 a) demonstrated that the transpiration rates of hardened seedlings in light and in the dark were similar to unhardened seedlings in the dark. For pine the transpiration rates were about 100 mg water per g dry matter needles and hour and for spruce 200 mg. Spruce and pine seedlings grown in nutrient solutions had similar transpiration rates in darkness (unpublished). Seedlings hardened for three weeks in the present investigation had transpiration rates in light (Table 6, 7, and 8) of about 700—900 mg, showing that the stomata were not closed. From these results it can be supposed that a hardening period of three weeks may influence the stomata mechanism to some extent, or the capacity of the water transport system. The effect on transpiration rates seems to be continuous: three weeks hardening decreases the transpiration rate by 50 per cent, but a hardening period of three months decreases it by almost 100 per cent.

Potassium transport

The analytical values (Table 3, 4, and 5) show ion antagonism phenomena demonstrated many times before for pine (see e.g. Ingestad 1960). However, the results of the present investigation also show the effect of a hardening period on the contents of some macronutrients in shoots and roots. A constant tendency for change in the content of potassium in the shoot and root during a hardening period was very clear (Table 3, 4, and 5). Shoots of hardened seedlings contained more potassium than shoots of unhardened seedlings. The increase in the amount of potassium in the shoot was equivalent to about half the decrease in potassium in the root. However, these values are related to dry matter, and the ratio between shoot and root dry matter is about 2, so that all the potassium lost from the root is found in the shoot. These results indicate that potassium ions are transported from the root to the shoot during a hardening period.

Potassium has been reported to increase winter survival (Levitt 1956, Benzian et al. 1966, Burgtorf 1968), but the results of the present investigation indicate that the effect on frost hardiness of potassium content is very small (Table 9 and 10). In this connection it is of interest to discuss the transport of potassium from the root to the shoot during a hardening period and the decrease in transpiration rate at increased potassium contents. It is supposed that one part of winter survival is an avoidance of winter dryness (Ebermann 1873). The decrease in transpiration rate at increased potassium content in the needles is rather small in the present experiment and the influence on survival within the investigated concentration ranges is very doubtful. According to Tamm (1964, page 146) the critical content for potassium deficiency is 0.075 mmoles per g dry matter, and such low contents have not been investigated in the present experiments (Table 3). Blanchet et al. (1962, reviewed by Mengel 1968, page 324) reported very high transpiration rates for potassium-deficient plants of lucern. If there are similarly high transpiration rates in potassium-deficient pine seedlings, and in some cases the seedlings in the forest and nurseries suffer to some extent from potassium deficiency, the transport of potassium from the root to the shoot during a hardening period is probably of great importance for the transpiration rates of the seedlings and may well be one of many reactions of the plants to increase winter survival.

Several authors have observed that potassium favours many physiological processes in plants, especially in metabolism and in transport mechanisms (see e.g. Haeder and Mengel 1972). The transport of potassium from root to shoot during the hardening period can be regarded as a defensive reaction of the plant, as it is well-known that the shoots are exposed to much lower temperature and severe stress than the roots during the winter (e.g. Bauer et al. 1971).

Frost hardiness

Frost hardiness development is considered to take place in stages (Tumanov 1967, Weiser 1970), where short days and low temperature govern the development. In the present investigation the seedlings were hardened for only three weeks, so that it is only the initial phase of frost hardiness development that has been investigated.

In considering the results of the frost hardiness determination, it is necessary to remember that the difference between two test temperatures in the method used was 0.3°C for unhardened and 1.0 for hardened seedlings, which is smaller than in most other similar investigations (Krasavtzev 1968, Schwarz 1968, Aronsson and Eliasson 1970, Christersson and Krasavtzev 1972), in which the difference varied between 2 and 10°C. When using such an accurate method, the heterogenity of the material is clearly shown (Table 9) and should be borne in mind when interpreting the results (Tables 9 and 10).

One weakness of the method is that the estimate of injury is subjective. Other authors have used objective methods (for references, see Aronsson and Eliasson 1970) in which it is possible to measure whether the tissues have been injured by frost and also the degree of injury. However, in the present investigation the intention was to determine seedling survival, which was possible as the method allowed the use of intact plants. After only a week it was possible to decide whether pine seedlings were dead or not. No damage intermediate between the brown tips of a few needles and more than half the needles brown could be distinguished. In the first group the xylem was white and in the second it was brown, so the discontinuity in the degree of injuries probably depended upon whether or not the xylem was injured (Potter 1939). A seedling with brown xylem after one week was always dead after 2 months. A seedlings with white xylem after one week was growing after 2 months.

The results indicate that different potassium, calcium, and magnesium contents in the shoots of unhardened and 3 weeks hardened seedlings of pine have no effect or only a small effect on the initial phase of frost hardiness developemnt. It is assumed that the ratio between potassium and calcium concentrations in the cells partly determines the water permeability of membranes (see e.g. Nason and McElroy 1963). In the literature it is supposed that high frost hardiness is correlated with high permeability for water in the cell membranes (Levitt 1966, Kuiper 1967, Mazur 1969). In the present investigation the ratio between potassium and calcium on a molar basis, in shoots of pine seedlings varies from 12 to 2, so that, if these theories are correct, the different nutritional treatments should have resulted in differences in water permeability and consequently in frost hardiness. But the results of the frost hardiness tests do not support these ideas.

Practical applications

It is necessary to be very cautious with suggestions for practical applications, as the work consisted of indoor experiments and included only a broad spectrum of interesting concentrations. However, the results indicate that, within the range of maximum or almost maximum growth rates, it is not possible to change the initial phase of development of frost hardiness of Pinus silvestris seedlings by changing the internal contents of potassium, calcium, and magnesium. A better winter survival may be achieved by an increase of the internal potassium content, since potassium is assumed to improve the water economy of the seedlings. The potassium content can be increased to a value as high as 0.4—0.5 mmol per g dry matter without causing much decrease in growth rate.

Acknowledgements

The author is very grateful to Professor Hans Burström for valuable discussions and criticism during the preparation of the manuscript. To the initiator of the investigation, Professor T. Ingestad, The Royal College of Forestry, Stockholm, Sweden, I wish to express my sincere thanks for his never-failing interest and for all suggestions and impulses that he has given me. It is a pleasure to acknowledge the technical assistance of Miss Ann-Margret Svensson.

The investigation was supported by a grant from The National Council for Forestry and Agricultural Research in Sweden.

Sammanfattning

Inom det svenska skogsbruket bedrivs för närvarande en intensiv forskning angående möjligheterna att snabbt och ekonomiskt framställa ett plantmaterial av hög kvalitet. I dessa försök har man utnyttjat den möjlighet som plastväxthus erbjuder att optimera en rad för växterna betydelsefulla faktorer såsom koldioxidkoncentrationen, vatten- och mineralämnesnivåerna. Det har emellertid framförts farhågor för att en på detta sätt åstadkommen hög tillväxthastighet skulle medföra en försämrad frost- och torkhärdighet.

Från fältförsök är det känt att god tillgång på kalium förbättrar barrträdsplantors vinteröverlevnad. Man vet dessutom att cytoplasmans svällningstillstånd och cellmembranernas permeabilitet för vatten, båda synnerligen väsentliga faktorer för utveckling av frost- och torkhärdighet, till en del regleras av cellernas innehåll av kalium och kalcium.

Syftet med den föreliggande undersökningen har varit att utreda huruvida det är möjligt att med bibehållen hög tillväxthastighet variera innehållet av kalium, kalcium och magnesium i skott och rot hos unga tallplantor på ett sådant sätt att plantor med god förmåga till frost- och torkhärdighet erhålles. Initiativet till undersökningen har tagits av professor T. Ingestad, Skogshögskolan, Stockholm, och ett mycket nära samarbete har etablerats under undersökningens gång med olika institutioner vid Skogshögskolan. Undersökningen ingår i ett större forskningsprogram, som pågår vid Skogshögskolan, och som syftar till att utreda mineralämnes- och gödslingsbehovet för hög produktion inom det svenska skogsbruket.

Tallplantor har odlats i vattenkulturer med varierande innehåll av kalium, kalcium och magnesium. Koncentrationsnivåerna av dessa tre joner har valts på ett sådant sätt att hög tillväxthastighet erhållits och att denna hastighet endast i ringa utsträckning påverkats av variationerna i de tre jonernas koncentrationer. Efter en tillväxtperiod på 12 veckor har hälften av plantmaterialet använts för experimentell bestämning av vatteninnehåll, transpirationsintensitet och frosthärdighet, medan den andra hälften har härdats vid kort dag och låg temperatur i tre veckor innan denna del har undersökts på motsvarande sätt.

Av resultaten framgår att en härdningsperiod på tre veckor minskar vatteninnehållet i skottet med 1 till 6 procent och transpirationsintensiteten med ca 50 procent. Ökat kaliuminnehåll medför hos både härdade och icke härdade plantor ett högre vatteninnehåll i skottet, medan transpirationsintensiteten minskar. Ett högre magnesiuminnehåll ökar vatteninnehållet, men påverkar ej transpirationsintensiteten. Resultaten från kalciumserierna tyder på att ökat innehåll av denna jon icke har någon signifikant verkan varken på vatteninnehåll eller transpirationsintensitet.

Resultaten av frosthärdighetsundersökningarna visar att olika halter av kalium, kalcium och magnesium i skottet har mycket liten effekt på frosthärdigheten hos icke härdade eller hos tre veckor härdade tallplantor.

Analysresultaten ger vid handen att kaliumhalterna, räknade på torrvikten, i härdade skott överstiger halterna hos icke härdade skott samt att det omvända förhållandet råder i roten. Dessa resultat antyder således en transport av kalium från roten till skottet under härdningens initialfas.

De sistnämnda resultaten är intressanta att diskutera i samband med publicerade resultat från fältförsök, som redovisar en klar effekt av kalium på vinteröverlevnaden hos barrträdsplantor. Här föreliggande undersökning visar emellertid att kaliumhaltens inverkan på själva frosthärdigheten är synnerligen ringa. För att förklara kaliumjonens inverkan på vinteröverlevnaden bör därför transpirationsintensiteten ägnas större uppmärksamhet. Resultaten i denna undersökning visar att en viss minskning i transpirationsintensitet åtföljer den ökade kaliumhalten i skotten. Transporten av kalium från roten till skottet under härdningsfasen skulle därför kunna ses som en reaktion av många som pågår i växten under frosthärdighetsfaserna, och som medför ökad kaliumhalt i härdade skott, vilket i sin tur innebär minskad transpirationshastighet. Ökad kaliumhalt i skottet kan därför antas spela en viss roll för plantors förmåga att undvika letala vattenpotentialer under t.ex. vårvintern, då höga temperaturer kan uppkomma i barren med relativt hög transpirationsintensitet som följd, och då tillgången på vatten för rötterna är starkt begränsad av tjäle i marken.

References

Aldén, T. 1971. Influence of CO_2, moisture and nutrients on the formation of Lammas growth and prolepsis in seedlings of Pinus silvestris L. — Stud. Forest. Suec. 93: 1—21.

Alden, J. & Hermann, R. K. 1971. Aspects of the cold-hardiness mechanism in plants. — 1: 37—142.

Arland, A. & Enzmann, J. 1956. Ein Beitrag zur Technik der „Anwelkmethode". — Zeitsch. Acker- und Pflanzenb. 101: 5—21.

Aronsson, A. & Eliasson, L. 1970. Frost hardiness in Scots pine. — Stud. Forest. Suec. 77: 3—30.

Bauer, H., Harrasser, J., Bendetta, G. & Larcher, W. 1971. Jahresgang der Temperaturresistenz junger Holzpflanzen im Zusammenhang mit ihrer jahreszeitlichen Entwicklung. — Ber. Dtsch. Bot. Ges. 84: 561—570.

Benzian, B., Bolton, J., Coulter, J. K. & Mattingly, G. E. G. 1966. Nutrition experiments in forest nurseries. — Extr. from Rothamsted Ann. Report 99—102.

Biebl, R. 1958. Der Einfluss der Mineralstoffe auf die Transpiration. — Handb. der Pflanzenphysiol. IV: 382—426.

Blanchet, R., Studer, R. & Chaumont, C. 1962. Quelques aspects des interactions entre l'alimentation potassique et l'alimentation des plantes. — Ann. agron. 13: 93—110.

Brag, H. 1972. The influence of potassium on the transpiration rate and stomatal opening in Triticum aestivum and Pisum sativum. — Physiol. Plant. 26: 250—257.

Burgtorf, H. 1968. Beispiele von Kaliummangel bei Fichten, gepflanzt auf Ackerboden. — Res. Not. Coll. of Forestry, Sweden 7: 1—19.

Burström, H. G. 1968. Calcium and plant growth. — Biol. Rev. 43: 287—316.

Christersson, L. 1971. Frost damage resulting from ice crystal formation in seedlings of spruce and pine. — Physiol. Plant. 25: 273—278.

— 1972 a. The transpiration rate of unhardened, hardened, and dehardened seedlings of spruce and pine. — Physiol. Plant. 26: 258—263.

Christersson, L. & Krasavtzev, O. A. 1972. Effect of velocity and duration of cooling on the frost hardiness of conifers. — Sovj. Plant Physiology 19: 638—642.

Ebermayer, E. 1873. Die physikalichen Einwirkung des Waldes auf Luft und Boden. — Bd. 1: 251—261. Aschaffenburg.

Elowsson, S. & Perttu, K. 1970. Measurements of water content in low humified peat by gravimetric methods and microwave technics. — Res. Not. Coll. of Forestry, Sweden. 22: 1—21.

Fisher, R. A. 1968. Stomatal opening: Role of potassium uptake by guard cells. — Science 160: 784—785.

Fujino, M. 1967. Role of adenosine-triphosphate and adenosine-triphosphatase in stomatal movement. — Sci. Bull. Fac. Educ. Nagasaki Univ. 18: 1—47.

Haeder, H. E. & Mengel, K. 1972. Translocation and respiration of assimilates in tomato plants as influenced by K nutrition. — Zeitsch. f. Pflanzenern. u. Bodenk. 131: 139—148.

Humble, G. D. & Raschke, K. 1971. Stomatal opening quantitatively related to potassium transport. — Plant Physiol. 48: 447—453.

Ingestad, T. 1960. Studies on the nutrition of forest tree seedlings. III Mineral nutrition of pine. — Physiol. Plant. 13: 513—533.

— 1962. Macro element nutrition of pine, spruce, and birch seedlings in nutrient solutions. — Medd. från statens Skogsforskningsinst. 51: 7.

— 1970. A definition of optimum nutrient requirements in birch seedlings. I. — Physiol. Plant. 23: 1127—1138.

— 1971. A definition of optimum nutrient requirments in birch seedlings. II. — Physiol. Plant. 24: 118—125.

Keller, T. 1967. The influence of fertilization on gaseous exchange of forest tree species. — Proc. colloq. on forest fertiliz. Jyväskylä, Finland. 65—79.

Kozlowski, T. T. & Clausen, J. J. 1965. Changes in moisture contents and dry weights of buds and leaves of forest trees. — Bot. Gaz. 126: 20—26.

Kramer, P. J. 1942. Species differences with respect to water absorption at low soil temperatures. — Am. J. of Bot. 29: 828—832.

Krasavtzev, O. A. 1968. Über die Gefriervorgänge bei pflanzlichen Gewebe. — Tagungsb. 100: 23—34.

Kuiper, P. J. C. 1967. Surface-active chemicals as regulators of plant growth, membrane permeability and resistance to freezing. — Med. Landbouwhogeschool, Wageningen, Nederland 3: 1—23.

Langlet, O. 1936. Studien über die physiologische Variabilität der Kiefer und deren Zusammenhang mit dem Klima. — Medd. Stat. Skogsförsöksanst. 29: 219—470.

Levitt, J. 1956. The Hardiness of Plants. — New York.

— 1966. Winter hardiness in plants — In Cryobiology. (H. T. Meryman, ed.) Academic Press. New York.

Little, C. H. A. 1970. Seasonal changes in carbohydrate and moisture content in needles of balsam fir (Abies balsamea). — 48: 2021—2028.

Lyr, H., Polster, H. & Friedler, H. J. 1967. Gehölzphysiologie. — Jena.

Marinos, N. G. 1962 a. Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. — Am. J. Bot. 49: 834—841.

Marschner, H. 1969. Mineralstoffwechsel. — Fortsch. der Bot. 31: 87—99.

Mazur, P. 1969. Freezing injury in plants. — Ann. Rev. Plant Physiol. 20: 419—448.

Mengel, K. 1968. Ernährung und Stoffwechsel der Pflanze. — Jena.

Nason, A. & McElroy, W. D. 1963. Modes of action of the essential mineral elements. — In Plant Physiology. (F. C. Steward, ed) III: 451—536.

Olsen, R. & Diehl, H. 1963. o,o'—Dihydroxyazo compounds as fluorometric reagents for magnesium. — Analytical Chem. 35: 1142—1144.

Parker, J. 1949. Effects of variations in the root—leaf ratio on transpiration rate. — Plant Physiol. 24: 739—743.

Potter, G. F. 1939. Low temperature effects on woody plants. — Proc. Am. Soc. Hort. Sci. 36: 185—195.

Raschke, K. & Fellows, Margaret. 1971. Stomatal movement in Zea mays: Shuttle of potassium and chloride between guard cells and subsidiary cells. — Planta 101: 296—316.

Sawhney, B. L. & Zelitch, I. 1969. Direct determination of potassium ion accumulation in guard cells in relation to stomatal opening in light. — Plant Physiol. 44: 1350—1354.

Schwarz, W. 1968. Der Einfluss der Temperatur und Tageslänge auf die Frosthärte der Zirbe. — Tagungsb. 100: 55—63.

Sirén, G. 1968. Synpunkter på skogsodlingens mekanisering. Skogs- o. Lantbr.-akad. Tidskr. 107: 271—289.

Stålfelt, M. G. 1956. Die stomatäre Transpiration und die Physiologie der Spaltöffnung. — In Handb. der Pflanzen-physiol. III: 351—426.

Tamm, C. O. 1964. Determination of nutrient requirements of forest stands. — Intern. Rev. Forestry Res. 1: 115—170.

Tumanov, I. I. 1967. The frost-hardening process of plants. — In The Cell and Environmental Temperature (A. S. Troshin, ed). Pergamon Press. Oxford.

Turner, N. C. 1972. K^+ uptake of guard cells stimulated by fusicoccin. — Nature 235: 341—342.

Van Steveninck, R. F. M. 1965. The significance of calcium on the apparent permeability of cell membranes and the effects of substitution with other divalent ions. — Physiol. Plant. 18: 54—69.

Weiser, C. J. 1970. Cold resistance and injury in woody plants. — Science 3952: 1269—1278.

Willmer, C. M. & Mansfield, T. A. 1970. Further observation of cation-stimulated stomatal opening in isolated epidermis. — New Phytol. 69: 639—645.

Youngburg, G. E. & Youngburg, M. V. 1930. Phosphorus metabolism. I. A system of blood phosphorus analysis. — J. Lab. Clin. Med. 16: 158—166.

Zech, W. & Franz, F. 1969. Nettoassimilation und Transpiration von Kiefernzweigen in Abhängigkeit von Kaliumversorgung und Lichtintensitet . — Forstwiss. Centralblatt 6: 372—378.