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Growth Rhythm and Frost Hardiness Dynamics in Norway Spruce (*Picea abies* (L.) Karst.)

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Populations of southern origins tended to initiate growth and dehardening later in spring, and start growth cessation and hardening later in autumn, than populations of orthern origins. Populations transferred more than approximately 3° in latitude showed poor growth performances due to lower numbers of stem-units. Southern populations showed prolonged apical mitotic activity compared with those of northern and local origins.

Progenies of selected plus-trees showed a later start of growth and slightly later dehardening in spring. Growth cessation occurred later in juvenile seedlings of selected populations than in natural populations of similar origin. Furthermore, in non-juvenile trees of selected populations prolonged mitotic activity was observed. Needle frost hardiness levels in selected populations were similar to those of natural populations of similar origin. Selected populations of northern origins tended to produce more stem-units than natural populations of similar origin.

Throughout the studies, variation in duration of mitotic activity appeared to be unrelated to the number of stem-units produced. This was evident both among populations and among clones of similar origins. Furthermore, variation in the ability to produce stem-units could not explain variation in accumulated height growth among natural populations. Growth and hardiness performances of southern populations and of selected populations of local origin appeared, at least in part, to have a similar physiological basis i.e. delayed spring and autumn phenology.

Key words: buds, frost hardiness, growth, mitotic activity, needles, Norway spruce, *Picea abies*, stem-units

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Terminology

Acclimation
Morphological and physiological adjustment by individual plants to compensate for the decline in performance following exposure to unfavourable levels of one environmental factor (Lambers et al.1998)

Adaptation
Genetically determined trait that enhances the performance of an individual in a specific environment (Lambers et al.1998)

Apical meristem
Meristem at the apex of an embryonic shoot. Commonly divided into four cytohistological zones; the peripheral zone, the apical initial zone at the summit of the apex, the central mother cells zone below the apical initials and the rib meristem zone further below.

Cell cycle
Sequence of events that includes a period of DNA synthesis (S), preceded by the first gap phase (G1) and followed by the second gap phase (G2), and mitosis (M) (Steeves & Sussex 1989).

Crown
The living tissue situated below the embryonic shoot consisting of cell with irregularly thickened non-lignified cell walls with many tannin filled pits (Romberger 1963, Venn 1965, Hejnowicz & Obarska 1995). Also called "nodal diaphragm".

Dormancy
The condition in which no cell divisions occur in meristematic tissues (Romberger 1963, Owens 1968).

Differentiation
The changes that occur in cells and groups of cells, and bring about their distinctiveness (Steeves & Sussex, 1989).

Early test
Evaluation of a juvenile trait in order to predict a mature trait.

Extracellular freezing
Ice formation on the surface of the cell or between the protoplast and the cell wall (Sakai & Larcher 1987).

Extraorgan freezing
Ice segregation from a supercooled organ to a specific space outside, resulting in dehydration of the organ (Sakai & Larcher 1987).

Free growth	Initiation and elongation of shoot primordia in young seedlings without a period of dormancy in between. Sylleptic free growth starts immediately after predetermined growth and proleptic free growth starts after a temporary bud has been set (Wühlisch & Muhs 1986).
Freezing avoid-ance	The ability of living cells to prevent (avoid) the establishment of thermodynamic equilibrium with the frost stress (Levitt 1980)
Freezing injury	Damage connected with ice formation in plant tissues (Sakai & Larcher 1987)
Freezing point	The temperature at which a liquid becomes a solid (Encyclopædia Britannica 1992)
Freezing resis-tance	The ability of cells to survive frost by means of avoidance or tolerance of ice formation in plant tissues (Levitt 1980)
Freezing tolerance	The ability of living cells to resist internal freezing stress without suffering injury (Levitt 1980)
Frost hardiness	Physiological condition that allows exposure to subzero temperatures without cellular damage (Lambers et al. 1998)
Growth	Irreversible increase in size accomplished by a combination of cell division and cell enlargement (Steeves & Sussex, 1989).
Intercalary meris-tem	Growth zone at the base of an elongating internode (or equivalent) that may function as a persistent isolated meristem bounded both above and below by mature tissues (Steeves & Sussex, 1989).
Intracellular freezing	Ice formation within the protoplast (protoplasm, vacuole) (Sakai & Larcher 1987).
Juvenile shoot growth	Shoot growth occurs in the way of free and predetermined growth (Wuehlish & Muhs 1991)

Meristem   Region of cells capable of division and growth in plants (Encyclopædia Britannica 1992)

Mitosis   A process of cell duplication, or reproduction, during which
                  one cell gives rise to two genetically identical daughter cells.
                  Mitosis is often subdivided into four phases: prophase, metaphase, anaphase and telophase. (Encyclopædia Britannica
                  1992)

Mitotic Index (MI) The percentage of nuclei in mitosis (Clowes 1960).

Natural population Progenies of open-pollinated trees where the trees have been
                  randomly selected in an autochthonous natural stand. (Similar
                  to the "ecotype"-term defined by Lambers et al.(1998).)

NSU   Number of stem units (Bongarten 1985).

Pith rib-meristem Meristem along the shoot axis of the elongating embryonic
                  shoot, previously formed by the rib-meristem of the apical
                  meristem, which results in elongation of stem-units.

Plus-tree   An individual tree phenotypically selected for its superior accumulated height growth compared to reference trees in the
                  same stand.

Population   Provenance, ecotype or group of plus-trees with a defined origin

Predetermined   Elongation of stem and needle primordia that were initiated
growth   during the previous year (Pollard & Logan 1974)

Seed orchard   Clonal composition of vegetatively propagated plus-trees established at a certain location in order to produce genetically
                  improved seed of high quality.

Selected popula- Progenies of open-pollinated plus-trees established in a seed
tion   orchard.

Stem-unit   Needle + internode (Doak 1935)

Supercooling   The cooling of a liquid below its freezing point without freezing taking place (Sakai & Larcher 1987).

Contents

Appendix

The thesis is based on studies reported in the following articles, which are referred to in the text by the corresponding Roman numerals.

I.   Westin, J., Sundblad, L.-G. and Hällgren,J.-E. 1995. Seasonal variation in photochemical activity and hardiness in clones of Norway spruce, *Picea abies* L. Tree Physiol. 15: 685-689.

II.   Westin, J., Sundblad, L.-G., Strand, M. and Hällgren, J.-E. 1999. Apical mitotic activity and growth in clones of Norway spruce in relation to cold hardiness. Can. J. For. Res. 29: 40-46.

III. Westin, J., Sundblad, L.-G., Strand, M. and Hällgren, J.-E. 2000. Phenotypic differences between natural and selected populations of *Picea abies* I. Frost hardiness. Scand. J. For. Res. (Accepted)

IV.   Westin, J., Sundblad, L.-G., Strand, M. and Hällgren, J.-E. 2000. Phenotypic differences between natural and selected populations of *Picea abies* II. Apical mitotic activity and growth related parameters. Scand. J. For. Res. (Accepted)

V.   Hannerz, H. and Westin, J. 2000. Growth cessation and autumn frost hardiness in one-year-old *Picea abies* progenies from seed orchards and natural stands. Scand. J. For. Res. (Accepted)

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Introduction

Seed sources, growth rhythm and frost hardiness

Growth rhythm and frost hardiness dynamics are important traits, with a strong influence on growth performance in boreal climates. Through the on-going process of adaptation, many tree species have developed natural populations that show enhanced fitness under specific environmental conditions. In Norway spruce (*Picea abies* (L.) Karst.) the origin of the seed source is known to influence various aspects of growth rhythm and development of frost hardiness. Therefore, in artificial reforestation the origin of the seed source may influence growth.

There are three types of seed source origin for Norway spruce. They may be: 1. local seed sources collected from trees with similar origins to the planting site, 2. transferred seed sources collected from trees of a different origin than the site (i.e. differing in latitude or altitude), 3. seed sources from clonal seed orchards composed of plus-trees, selected for superior height performance. The third alternative may be subdivided into specific seed sources produced on trees that have been transferred to environments more or less different from the original environment.

Transferred natural stand seed of Norway spruce and seed produced in seed orchards are commonly used in Swedish forestry. In Sweden transferred natural stand seed has been used on a large scale since the 1950's and seed orchard seed from the 1960's. The use of seed from non-local sources is motivated not just by seed quality considerations, but also by its potential to establish highly productive stands (Anon. 1993).

The migration history of Norway spruce after the latest glaciation involves a northward migration of spruce, from refuges in Central and Eastern Europe, to northern Fennoscandia (Schmidt-Vogt 1977). Further migration in Scandinavia occurred mainly from north to south. It is commonly stated that the migration pattern of spruce in Fennoscandia has affected growth rhythm and hardiness and that the present clines in these characters are remnants of the migration history.

When natural Norway spruce populations with different seed origins are evaluated at a set location, seed sources transferred northward show increased height growth (Worrall 1975, Skrøppa and Magnussen 1993), and later flushing (Worrall 1975) with similar survival rates (Rosvall & Eriksson 1981) compared with populations of local origin. Similarily, progeny of selected plus-trees tend to show higher growth potential (Johnsen 1989, Skrøppa & Johnsen 1999, Karlsson 1999) with the same survival rates as compared with local seed sources.

The main objective with of the work reported in this thesis was to study the seasonal rhythm of growth and hardiness in order to characterise the dynamics of growth and hardiness

in local, transferred and selected seed sources.

Shoot growth in conifers

Free growth and predetermined shoot growth

Shoot growth can be either predetermined or free. In the genera *Picea*, one–year old seedlings display only free sylleptic shoot growth, whereas older seedlings can show predetermined shoot growth and various amounts of free shoot growth (sylleptic or proleptic) (Wuehlish & Muhs 1986). The ability to display free growth is a juvenile feature that gradually decreases as the plant becomes older (Pollard et al.1975). Free growth can be regarded as an independent contribution to juvenile height growth which will be lost with age (Pollard & Logan 1974) or as precocious height growth that will be replaced by predetemined growth of a similar magnitude with age (Cannell & Johnstone 1978, Ununger & Kang 1988). Free growth and predetermined growth appear to be two forms of shoot growth that are well integrated and are not inherited independently of each other (Wuehlish & Muhs 1991).

The amount of free growth is strongly influenced by environmental conditions, especially short-days (Dormling 1968) and fertilisation (Wuehlish & Muhs 1991). Free growth is not influenced by the amount of preceding predetermined growth, but it has positive effects on the following predetermined growth as well as on the initiation of needle primordia (Ununger & Kang 1988, Wuehlish & Muhs 1991). Free growth is also influenced by genetic origin, as some provenances have a higher potential for free growth on favourable sites than other provenances (Pollard & Logan 1974, Cannell & Johnstone 1978, Wuehlish & Muhs 1991).

Shoot meristems

Shoot anatomy and bud morphology of most genera, and many species within the Pinaceae, have been thoroughly described and show strong similarities. A common zonation pattern exists within the apex although differences occur in the absolute size of the apex and the relative size of the cytological zones between individuals and species (Burley 1966). In the genera *Picea* and *Abies* a crown exists in the anatomy of the buds (Parke 1959, Romberger 1963), which appears to be a feature of some importance in the process of extra-organ freezing.

The initiation and development of primordia in the apical meristem have previously been described in *Pseudotsuga menziesii* by Owens (1968), in *Picea glauca* by Owens et al. (1977) and in *Picea abies* by Hejnowicz & Obarska (1995). Generally, budscales and needle primordia are initiated in the peripheral zone of the apical meristem. A change from bud-scale initiation to needle initiation occurs at the completion of shoot elongation. The initiated primordia enlarge, after an initial period of division in all planes, through divisions in an intercalary

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meristem and by cell enlargements throughout the primordia. The number and shape of all foliar organs initiated in buds of mature trees can generally be determined before dormancy.
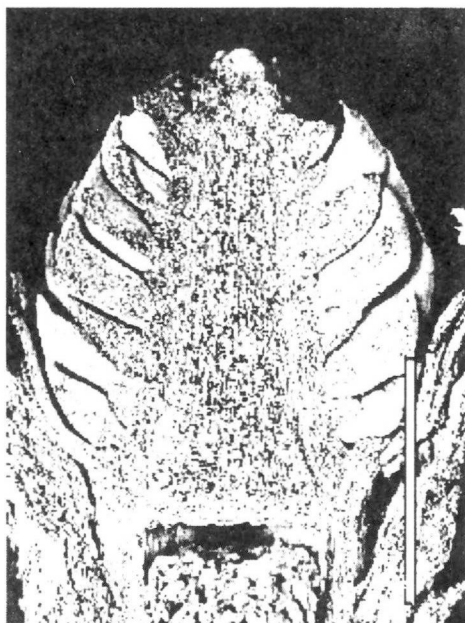

Fig. 1. Embryonic lateral shoot of *Picea abies* (origin 60°35'N) collected in a field test at Sävar (63°54'N, 20°33'E, alt. 10 m) on June 2, 1997. At the top is the apex, in the middle are elongating preformed needles and the pith rib-meristem, and below these is the crown. The vertical line indicates 1 mm. (Courtesy P. Hörstedt, Dept. of Pathology, Univ. of Umeå, Swe den).

After dormancy the bud scales generally undergo no noticeable enlargement, but the needle primordia begin to mature at different times and at different rates. Maturation occurs basipetally and starts before the needles are fully elongated, simultaneously with divisions in the intercalary meristem. Maturation of needles continues throughout the entire growing season and is promoted by warm temperatures. The degree of maturation of needles affects various features, for example, the thickness of the needle cuticle, which may increase the ability of the needle to withstand drought stress in winter (Tranquillini 1979, Vanhinsberg & Colombo 1989).

Fig. 2. Apex of a terminal lateral shoot
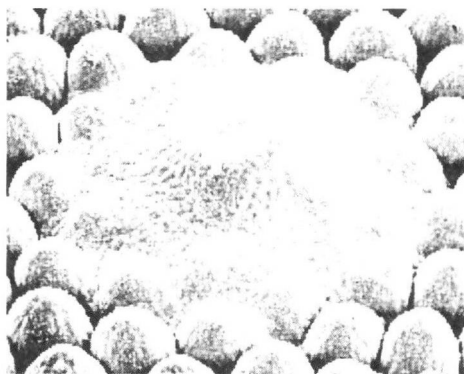
in *Picea abies* (Clone 115, origin 63ºN) collected in a field test at Sävar (63°54'N, 20°33'E, alt. 10 m) in November 1995. (Courtesy P. Hörstedt, Dept. of Pathology, Univ. of Umeå, Sweden).

The developmental stages as indicated by the morphological development before and after dormancy, are not definite. For instance, in mature trees of *Picea abies*, bud scales may be initiated both in late autumn and in spring (Hejnowicz & Obarska 1995). In one-year old seedlings of *Picea sitchensis*, with delayed bud development in autumn, a small number of needle primordia may also be initiated during the first part of the elongation phase in spring (Burley 1966).

Furthermore, developed organs

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may be transformed, as bud scale primordia may elongate to from broad and flat needle-like structures during the change from sylleptic to proleptic free growth with increasing age (Wuehlish & Muhs 1986).

All initiated needle primordia are attached to the pith rib-meristem, through the peripheral zone, along the axis of the embryonic shoot. The role of cell division and cell elongation in lateral shoot elongation have previously been described *in Pseudotsuga menziesii* (Owens et al.1985) and in *Picea engelmannii* (Owens & Simpson 1988). Generally, the preformed undifferentiated pith cells in the pith rib-meristem start to divide and elongate after dormancy. Early shoot elongation before flushing results from a rapid increase in mitotic activity in the pith rib-meristem, whereas late shoot elongation after flushing results from cell elongation. Shoot elongation as a whole results in increased mean stem-unit length. In *Picea engelmannii*, elongation of the original pith cells has been found to account for less than 15% of the final lateral shoot length. The remaining increase in shoot length was due to cell divisions and similar elongation in the length of the resulting daughter cells. Considering the overall similarities in shoot anatomy within the Pinaceae, the original pith cells and daughter cells seem likely to make similar relative contributions to final shoot length in *Picea abies*.
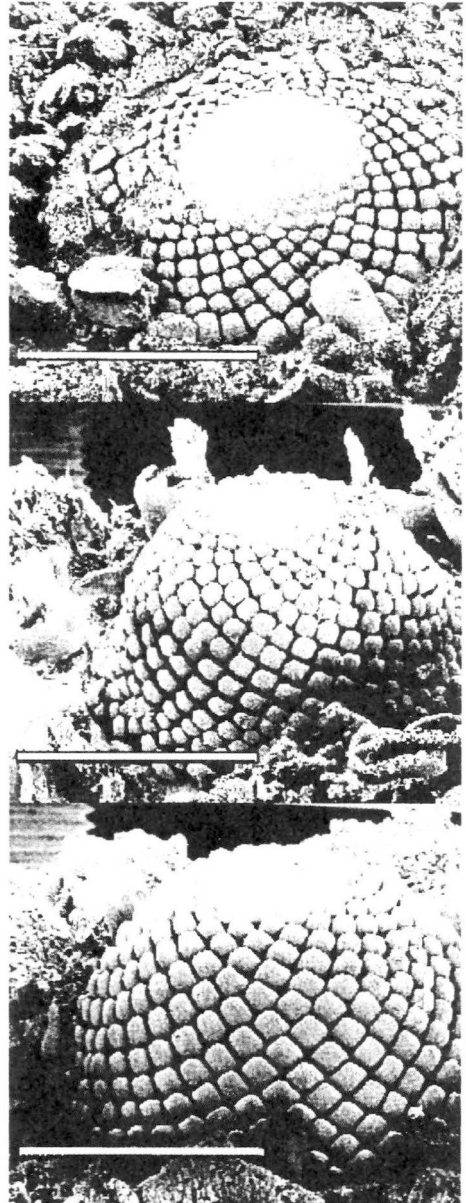

Fig. 3 a-c. Embryonic leader shoots of *Picea abies* (origin 63ºN) collected in a field test at Sävar (63°54'N, 20°33'E, alt. 10 m) on August 28 (top), September 30 (middle) and October 23 (below) in 1998. The horizontal lines indicate 1 mm. (Courtesy P. Hörstedt, Dept. of Pathology, Univ. of Umeå,, Sweden)

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Comparisons with the vascular cambium

The cells in the vascular cambium in conifers divide and differentiate into xylem and phloem. Initiation of cambial cell division in spring and its cessation in autumn are brought about both by internal chemical factors and external conditions (Savidge & Wareing 1981, Mellerowicz et al. 1992). In spring, cambial cell division appears to start in the living crown before or around bud-break (Savidge & Wareing 1984, Oribe et al. 1993). From the living crown, cell division appears to proceed both basipetally down the stem and acropetally to younger cambia (Savidge & Wareing 1984, Oribe et al. 1993). The pattern of cell division activity may reflect both internal and external factors. In many conifers, temperature is suggested to be a limiting external factor for cell division in spring, at least in older cambia (Savidge & Wareing 1981, Mellerowicz et al. 1992), whereas short photoperiods induce cessation of cambial activity in autumn (Mellerowicz et al. 1992). Indole-3-acetic-acid (IAA) appears to be a controlling internal factor for cambial growth, and is required by the vascular cambium for cambial cell division, radial enlargement and tracheary differentiation (Savidge & Wareing 1981, Little & Savidge 1987). Recent research has demonstrated that the supply of IAA polarly transported to the cambial tissues, and the resulting concentration and distribution pattern of IAA across these tissues is important in the control of cambial growth (Uggla et al. 1998, Sundberg et al. 2000).

Production of cells

Cell division in plants takes place in meristems, where cells pass through and between the different stages of the mitotic cell cycle. After cytokinesis, the cell passes through G1 and at a specific point late in G1, the fate of the cell is decided form one of four fates; to divide, arrest, differentiate, or senesce (Jacobs 1995). In addition to the decision point, checkpoints exist between the different phases where the conditions are checked before the cell cycle proceeds. Specific regulators control the checkpoints and the presence of these regulators in non-dividing plant cells may confer upon them mitotic competence. The developmental plasticity of plants suggests that an intermediate, developmentally metastable phase (G0), between proliferation and terminal differentiation, may exist in vegetative plant cells (Jacobs 1995).

The number of cells produced in a meristem during a year is dependent on four cell cycle parameters: the frequency of cells undergoing mitosis, the rate of cell division, the size of the meristem, and the duration of cell cycle activity.

The frequency of cells undergoing mitosis, as expressed by the Mitotic Index (MI), is the most frequently and readily used cell cycle parameter (Grob & Owens 1994). In mature trees, MI has been related to the developmental stage of vegetative buds

(Owens & Molder 1973) and water relations (Owens et al. 1985, Owens & Simpson 1988). Based on these relationships, and similar relationships occurring in seedlings, MI represents a powerful tool that can be used to describe cellular conditions in apical meristems throughout the seasonal growth cycle.

The relationships between MI and other cell cycle parameters are not consistent, and a change in MI does not necessarily indicate changes in factors such as the rate of cell division. For instance, in roots of *Zea mays* the length of the mitosis phase is not extended in proportion to the length of the cell cycle (Clowes 1960), whereas in roots of *Vicia faba* all stages of the cell cycle are reduced proportionally with lowered temperature (Murin 1981). In addition, cells in different zones of a meristem may show varying and independent rates of cell division and MI (Clowes 1960). Furthermore, it is obvious that large meristems tend to produce more cells than small meristems, but meristem size is in turn dependent on the rate of cell division and the frequency of cells undergoing mitosis.

The duration of cell cycle activity is species-dependent and varies between organs and tissues. In conifers with a distinct dormancy phase during winter, cell division resumes in the apical meristem in spring. In the embryonic shoot, cell division starts first in the needle primordia (Hejnowicz & Obarska 1995) then spreads to the peripheral zone, the pith rib-meristem and, lastly, the apex. In autumn, mi-

totic activity ceases first at the apex, then in needle primordia and internodal tissues, and finally in the youngest needle and bud scale primordia (Owens 1968, Hejnowicz & Obarska 1995). In the meristem of elongating organs, e.g. in needles and and the pith rib-meristem of shoots, cell division occurs as long as the organ elongates (Owens 1968, Hejnowicz & Obarska 1995).

Freezing resistance in conifers

The freezing process

To be able to withstand low winter temperatures, a sufficient frost hardiness level is essential. The development of freezing resistance is an active process, which takes place at a different pace in different organs. The freezing resistance of primordia and their survival mechanism are key problems in boreal conifers (Sakai & Larcher 1987). Generally, the freezing process of plant cells shows a common sequence of events as the temperature decreases. The main stages are supercooling to the ice-nucleation temperature, release of crystallisation heat and a subsequent rise in temperature to the freezing point, followed by coexistence of liquid and solid phases over a broad temperature range until a more or less solid phase is reached (Sakai & Larcher 1987).

In plant tissues the threshold temperature for ice-nucleation and the freezing point appear to be directly proportional to each other (Yelenosky & Horanic 1969). The phenomenon of

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supercooling is not fully understood, but the degree of supercooling appears to depend on cell size, relative water content and factors related to the presence or absence of ice-nuclei. In the absence of ice nuclei, pure water may supercool to -38.1°C (Sakai & Larcher 1987). In plants, supercooling represents a transient, unstable state, which may depress freezing 3-8°C below the freezing point, but in specific tissues, e.g. in floral primordia, much lower nucleation temperatures have been reported (Sakai & Larcher 1987). In addition, detached shoots and leaves appear to show a higher degree of supercooling and a lower freezing point than the intact plants.

Freezing point depression is exhibited in plants by an accumulation of sugars or other solutes in the cells (Ögren 1997, Ögren et al.1997) or by a decrease in water content.

Ice formation in plants occurs either intracellularly or extracellularly (Levitt 1980). Since intracellular freezing is considered to be lethal, the ability of plants to tolerate freezing in boreal conditions depends mainly on their ability to either tolerate the stress caused by extracellular (or extraorgan) freezing or by developing a state of supercooling. The rate of dehydration due to water migration is rapid in extracellular freezing, slow in extraorgan freezing and extremely slow or non-existent in supercooling (Sakai & Larcher 1987). Winter buds of the Abietoideae (*Abies, Picea, Tsuga, Larix, Pseudotsuga*) show extraorgan freezing whereas winter buds of the Pinoideae (*Pinus*) show extracellular

freezing.). The rate of water migration may affect the ability of a tissue to withstand sudden drops in temperature.

Ice normally crystallises in the water-conducting system, where the sap has the highest freezing point of any solution in the plant (Zimmermann 1964). Results from hardwoods show that when freezing is initiated, it proceeds from a few nucleation points along the xylem vessels and reaches all parts of a shoot within a relatively short time. (Sakai & Larcher 1987).

Freezing injuries

The plasma membrane appears to be the prime site of freezing injury (Steponkus 1984). The plasma membrane has a central role during freezing and thawing and functions both as a semi-permeable membrane for water diffusion through water channels (Steudle & Henzler 1995) and as a barrier for nucleation of the intracellular solution. In extraorgan freezing the membranes do not function as nucleation barriers either at the cellular level or in the organ, instead the barriers appear at the organ level or outside the organ e.g. the crown during freezing of the buds (Sakai & Larcher 1987). The membranes may experience various types of stress during freezing involving physical effects of the low temperature *per se*, freeze-induced solute concentration effects, freeze dehydration or changes in pH or ionic strength (Steponkus 1984, Hällgren & Öquist 1990).

Membranes of hardy cells are char-

acteristically resistant to penetration of ice crystals, and show high permeability to water. The extent of frost injury depends on lipid composition of the membranes and the presence of specific cryoprotectants (Hincha et al. 1990, Lin & Thomashow 1992, Nishida & Murata 1996). Furthermore, the extent of injury is also dependent on factors associated with the freeze-thaw cycle *per se*. The rate of cooling or thawing appears to be an important factor for the extent of frost injury, as the rate of diffusion of water through the membrane, to ice outside the cells, is limited by the permeability of the plasma membrane. High cooling rates may therefore result in non-equilibrium freezing followed by intracellular freezing. In freezing experiments, cooling rates of >10°C per min have been shown to cause needle damage, provided that freezing was initiated above -4°C and extended to below -10°C, (Perkins & Adams 1995).

Other important factors for frost injury associated with freeze-thaw cycles appear to be their duration and number and the post-thawing conditions. The minimum temperature levels occurring in nature appears not to be critical for plants in a state of winter-rest (Perkins et al. 1991, Strimbeck at al. 1993.).

Interactions between freezing and other factors effect the extent of frost injuries. For example, frost in combination with high levels of irradiance (Öquist & Strand 1986) and frost in combination with nutrient imbalances, particularly high concentrations of potassium (Perkins & Adams 1995) results in higher injury levels than frost alone. Depending on the extent of freezing injury and the importance of the damaged tissue, partial or even complete repair is possible (Sakai & Larcher 1987). Freezing injury weakens the plant and may temporarily reduce its potential to assimilate. Recovery from freezing injury is an active process, which requires available assimilates. Therefore, freezing injuries may not only involve direct losses of growth due to freezing, but may also a reduction in growth, due to competition for resources.

Seasonal variation in growth rhythm and frost hardiness in conifers.

Adaptation and acclimation to climate

Adaptation of growth rhythm and frost hardiness levels to seasonal variation in boreal climates is essential for survival and reproduction (fitness). Genetic variation among and within populations in the initiation and cessation of growth, and in the development of frost hardiness, is the basis of the process of adaptation. Individual plants may acclimate to various environmental conditions, and the feature may also be of major adaptive significance. The ability to acclimate is based on the structural and physiological plasticity of plants (Juntilla 1996) and the ability varies between different plant characteristics. Acclimation to different environmental

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conditions (e.g. temperature, photo-period and water stress) may result in similar responses in growth and frost hardiness levels.

Variation in initiation and cessation of growth

Data on cell division in conifers growing under natural conditions are scarce, and data on initiation and cessation of cell division has been mainly derived from studies on species grown under various conditions. Remarkable similarities in the timing of initiation and cessation of cell division are evident, even though different conifer species have been studied in a wide range of climates, e.g. in maritime climates (British Columbia, Canada) and continental climates (Poland). Generally, cell division in the apical meristem tends to start in late March to early April and ends in mid-October to late November. For example, cell division in the apical meristem starts in late March in *Pseudotsuga menziesii* (Owens 1968) and in *Pinus contorta* (O'Reilly & Owens 1987), in late March or early April in *Picea abies* (Hejnowicz & Obarska 1995) and in early April in *Picea glauca* (Owens et al.1977). Cell division in the apical meristem ends in mid-October in *Picea glauca* (Owens et al.1977) and in *Picea abies* (Hejnowicz & Obarska 1995), at the end of October in *Pinus contorta* (O'Reilly & Owens 1987) and in late November in *Pseudotsuga menziesii* (Owens 1968). However, in *Pinus taeda* cell division in the apical meristem has been found to continue all year, but at varying levels (Carlson 1985). Cell cycling in the vascular cambium of *Abies balsamea*, and subsequent differentiation into phloem and xylem, starts in May and ends in September (Mellerowicz et al. 1992).

Several environmental factors influence the level of apical mitotic activity, for example, photoperiod, temperature, nutrient-status and water availability. However, their influence on variation in initiation and cessation of growth is unclear. The initiation of growth is often assumed to depend primarily on the temperature requirement for initiation, whereas cessation of growth appears to be influenced by several factors. Both short-days and moisture stress have been found to reduce MI in *Tsuga heterophylla*, but in contrast to short days, moisture stress did not end cell division (O'Reilly et al. 1989). In studies on *Pinus taeda*, fertilisation temporarily affected MI levels but did not lead to changes in the timing of dormancy in the apical meristem (Carlson et al.1980, Williams & South 1992). It appears that photoperiod influences cessation of cell division in apical meristems, whereas the influence of temperature *per se* (Murin 1981) is less clear.

Variation in levels of frost hardiness

The seasonal variations in frost hardiness levels have been reported in various conifer species growing under natural conditions (Glerum 1976, Cannell & Sheppard 1982, Koski 1985, Repo 1992, Beuker et al. 1998). Generally, the level of frost hardiness tends to decrease from late March to

May, whereas it tends to increase from September to November. In many conifers freezing to -40°C or lower, during the winter, does not induce freezing injuries.

Temperature and photoperiod are the two main environmental factors that determine the level of frost hardiness (Glerum 1976). Several studies indicate that the dehardening occurs primarily as a response to increasing temperatures. However, photoperiod affects the timing of bud burst (Partanen et al. 1998) and effects of photoperiod on the level of frost hardiness can therefore not be excluded. Several studies indicate that hardening occurs in two or three stages (Weiser 1970, Glerum 1976). A short day stimulus appears to induce the first stage of frost hardiness development and subfreezing temperatures, just below 0°C, appear to induce the second step. A third stage induced by temperatures of −30 to -50°C has also been suggested. For buds and needles, fluctuations in the levels of frost hardiness during mid-winter appear to coincide well with fluctuations in ambient temperatures (Strimbeck et al. 1995, Beuker et al. 1998). In *Abies balsamea*, chilling temperatures, and (to a degree) short photoperiods promote development of frost hardiness of the cambium in autumn, whereas no effect of temperature and photoperiod has been observed in spring (Mellerowicz et al.1992).

Plants use light signals throughout their lifecycle to synchronise development with seasonal changes, thereby ensuring that the available resources are used effectively. Investigations at the physiological level indicate that phytochromes have roles in many ecological processes, for example induction of dormancy and frost hardiness development (Smith 1995, Olsen et al. 1997). The functions of the phytochromes in photoperiodic perception are the least well understood of the phytochrome functions. However, it seems to be established that phytochrome A has a role in long-day plants, and phytochrome B plays a role in the perception of short days. Results in hybrid aspen (*Populus tremula* × *tremuloides*) indicate that phytochrome A may be involved in the detection of photoperiod in trees (Olsen et al. 1997). Over-expression of phytochrome A has resulted in changes in the critical daylength and prevented cold acclimation. Furthermore, these changes were accompanied by changes in levels of several plant hormones e.g. gibberellins and IAA, which indicate that they may be involved in short-day induced growth cessation in trees.

Early testing of growth and frost hardiness

The purpose of growth rhythm and frost hardiness assessments in early-tests may vary, depending on the objectives of the tests. In tree breeding the focus is generally set more on the ranking of genotypes for specific traits, than on determining absolute values. Furthermore, the juvenile traits should reflect adaptive processes that are essential for survival and reproduction. Examples of juvenile traits

that reflect adaptive processes include freezing injuries after freeze-tests (Johnsen 1989), bud-set (Skrøppa 1988) and stem lignification (Pulkinnen 1993). As non-juvenile trees show only predetermined growth, juvenile traits in juvenile seedlings may not be directly related to the corresponding non-juvenile traits. For example the timing of bud-set is different on a one-year-old seedling compared with a non-juvenile tree.

A correlation between juvenile traits and mature traits is essential for valid early testing. In *Picea abies*, juvenile–mature correlations in growth cessation traits in autumn are often indirect and based on patterns among natural populations. For example, bud-set or hardiness in young seedlings follows the same latitudinal cline (Ekberg et al.1979) as autumn dry-matter content in older trees (Langlet 1960). Correlation estimates between traits that describe growth cessation in one-year old seedlings are usually strong at the population level (Johnsen & Apeland 1988, Pulkkinen 1993), whereas the estimates appear to be lower among families (Johnsen & Apeland 1988, Skrøppa 1991).

Testing of frost hardiness

In artificial freeze tests, plant samples (e.g. whole plants, detached shoots, buds or needles) are exposed to a series of low temperatures according to a defined freeze/thaw cycle. The result of the freezing injury evaluation after freezing is dependent on several factors. Factors associated with the freeze-thaw cycle have previously been discussed. In addition, the treatment of samples before and after freezing is important, as is the method used for assessing freezing injuries, e.g. measurement of Fv/Fm ratios, or electrolyte leakage.

Generally, frost hardiness levels should be considered as relative and not absolute, as the conditions before, during and after the freeze/thaw cycle are never exactly the same on different freeze occasions. Interpretation of the frost hardiness levels to specific temperatures (°C) should therefore be avoided, as the results are only valid under specific conditions set during the freeze test. Determination of frost hardiness levels is thus not different from determination of growth performances, which are often expressed in relative terms rather than absolute terms.

The method used for assessing freezing injuries should be carefully considered when interpreting them. Low Fv/Fm-ratios may indicate low photochemical efficiency caused by the freeze treatment. However, low Fv/Fm-ratios are also observed in acclimation to low temperature. Therefore, Fv/Fm-ratios near zero do not always indicate freezing injuries in photosystem (PS) II, as they may also be part of an important acclimation process to environmental factors.

Aim of the study

The most important aim of the study was to compare growth performance of selected populations of

local origin with that of southern natural populations, in order to clarify whether similarities in growth performances had similar physiological background. This was done by a characterising seasonal growth rhythms and seasonal frost hardiness development in local, transferred and selected seed sources. Additionally, the relative importance of various growth components and of frost hardiness for overall growth in Norway spruce was studied from a general perspective.

Results and discussion

Spring

Initiation of growth

In winter and early spring the levels of photochemical activity, as expressed by the Fv/Fm-ratio, were low in all populations studied, as an acclimation to low ambient temperature levels (**I, III**). In April, photochemical activity levels were further lowered in all populations to an seasonal minimum, which indicated photoinhibitory conditions presumably due to low night temperatures combined with excess light during the day (**I, III**). In late spring, photochemical recovery occurred in all populations, following the gradually increasing temperatures (**I, III**). The photochemical recovery in spring appeared to be mainly dependent on temperature, but an effect of population origin was also observed, as a slightly later recovery, or possibly a later onset, of photochemical activity was observed in some southern populations. Results are in accordance with Lundmark et al. (1998) and Berg & Linder (1999) where mean air temperature affected the photochemical recovery in spring. However, in their studies recovery was also affected by other factors, e.g. light conditions and frequency of severe night frosts.

In early spring (April) the levels of apical mitotic activity, as expressed by the mitotic index (MI), increased rapidly in all populations (**II, IV**). The high levels of mitotic activity appeared not to be influenced solely by temperature, as previous periods with relatively warm temperatures had no apparent effect on MI levels (**II, IV**). The results indicate a synchronous release of a cell cycle blockage. Previous studies of MI in *Pseudotsuga menziesii.*(Mirb.), *Franco* (Owens & Molder 1973) *Fraxinus excelsior* (Cottignies 1979) and *Abies balsamea* (Mellerowicz et al.1989) indicate that the cell cycle is blocked during autumn in the G1/S boundary.

After the short characteristic period in early spring, with high levels of apical mitotic activity in all populations, the activity levels decreased in all populations (**II, IV**). In mid spring, mitotic activity levels tended to increase again, following a gradual increase in temperature (**II, IV**). The mitotic activity levels appeared to be mainly dependent on temperature. However, an effect of population origin was also seen, as northern populations showed higher MI levels than southern populations (**IV**), indicating an earlier start of growth in the northern population. Furthermore, differ-

ences in MI levels indicated that growth started later in one of three clones of similar origin studied, than in the other two (**II**). The MI levels in spring appeared to reflect variation in temperature and population origins, but no effect of selection was detected. The results are in accordance with those of Cannell & Willett (1975) who showed that among both *P. sitchensis* and *P. contorta* populations, northern populations initiated apical meristematic activity before more southerly ones.

The start of shoot growth, as indicated by the date of bud burst, occurred earlier in northern populations than in southern populations (**IV**). An effect of selection on the start of shoot growth was also observed, as selected populations started shoot growth later than natural populations of similar origin (**IV**). Furthermore, among the three clones of similar origin studies, one showed a later start of growth than the other two (**II**). In 1996 and in 1997, shoot growth started on similar Julian days (Fig. 4), even though spring 1996 was warmer than spring 1997 (**III**). Furthermore, until the start of shoot growth, there were 74 chill days (≤ 5 °C from Nov. 1) in 1996, and 59 in 1997. The results indicate that the start of shoot growth was not influenced by observed differences in temperature sum (e.g. degree-days, > 5 °C) or in number of chill days. However, after shoot growth had started, further shoot growth development was mainly influenced by temperature. The data confirm results by Hannerz (1999), suggesting that chilling condi-

tions in southern and central Sweden are not limiting for bud burst. According to Worrall (1975) a variation in threshold temperatures for bud burst appears to exist, both among populations and among clones, and the ranking appears to be stable over the years.
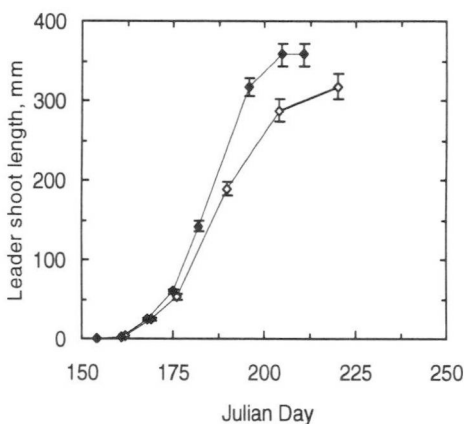

Fig. 4. Average leader shoot length for all populations in 1996 (◊) and in 1997 (♦). Standard error of the means are indicated by vertical bars.

It is possible that the observed differences in temperature sum in this study were not large enough to influence the overall start of shoot growth, and that populations may have different temperature requirements. Another possible explanation is that the start of shoot growth is also influenced by factors other than temperature. Partanen et al. (1998) showed that budburst in *Picea abies* was influenced by chilling temperatures and by the photoperiod in November and December. Heide (1993) showed that long days reduced the thermal time to bud burst at all flushing temperatures in some

northern decidous tree species (Heide 1993). Furthermore, Dormling (1982) found that well-hardened seedlings of Norway spruce flushed earlier than less hardy ones. Altogether, the start of shoot growth appeared to reflect genetic variation among populations and among clones, but not just through variation in characters such as temperature requirements.

Overall, the results from spring indicate a genetic variation in initiation of growth, both among populations and among clones of similar origin. Initiation of growth tended to start later in populations of southern origins than in those of northern origins, and differences in initiation of growth were observed among clones of similar origin. After growth had been initiated, further development of growth in spring was influenced by an increase in temperature. Variation among populations in initiation of growth could not be explained solely by different temperature requirements for initiation and, therefore, other factors appeared to be involved. Generally, the different parameters that were used to show different aspects of the dynamics of growth in spring appeared to give similar results, which indicate that they may be used interchangeably.

Dehardening

In spring, the frost hardiness levels, as expressed by the Fv/Fm-ratios after freezing and by relative conductivity (RC) ratios, gradually declined from late April to early May. Populations of northern origins dehardened slightly earlier than those of southern origins (**III**) and a selection effect was ob-

served, as selected populations appeared to deharden slightly later than natural populations of similar origin. Furthermore, among clones of similar origin a difference in frost hardiness levels in late spring/early summer was recorded (**I**). Generally, the process of dehardening appeared to be affected by temperature and possibly photoperiod, as a gradual increase in temperature resulted in decreased frost hardiness levels, whereas no dehardening occurred during warm periods in winter. In contrast to the Fv/Fm-results, the RC results indicated a simultaneous dehardening in northern and southern populations but a selection effect was observed, as selected populations appear to deharden later than natural populations of similar origin. The results are consistent with other studies (Sarvas 1972, Koski 1985, Repo 1992) that have shown dehardening to be influenced by temperature. However, they conflict with other studies on *Picea abies* (Beuker et al, 1998), *Picea glauca* (Simpson 1994) and *Picea sitchensis* (Cannell and Sheppard, 1982) in which no clear spring-time differences in hardiness between northern and southern populations were detected.

The results on dehardening in spring indicate a genetic variation in dehardening, both among populations and among clones of similar origin. In all populations dehardening tended to progress from very high hardiness levels in mid April down to low summer hardiness levels in late May or early June. Populations of northern origins

dehardened slightly earlier than populations of southern origins and a difference in dehardening between clones of similar origin was evident. Selection resulted in a slightly later dehardening in selected populations than in comparable natural populations. Variation among populations in initiation of dehardening could not be explained solely by differences in temperature requirements for dehardening. Thus, other factors appear to be involved. After dehardening had been initiated, further dehardening was promoted by increasing temperatures.

Conclusions regarding spring events

Taken together, the results on growth and dehardening in spring indicated genetic variation both among populations and among clones of similar origin. Initiation of growth and dehardening tended to start later in populations with southern origins than in populations with northern origins. Early initiation of growth appeared to be related to early dehardening among clones of similar origin. Furthermore, selection appeared to result in a later start of growth and a slightly later dehardening than in natural populations of similar origin. Variation among populations in initiation of growth and dehardening could not be explained solely by differences in temperature requirements, so other factors are likely involved. However, after growth and dehardening had been initiated, both processes were promoted by increases in temperature.

Autumn

Cessation of growth

In summer and early autumn all populations showed high photochemical activity, as expressed by the Fv/Fm-ratio, and the levels appeared to follow changes in ambient temperature (**I, III**). In populations of northern origin, the level of photochemical activity was lower from late autumn than in those of southern origins, but no selection effect on the level of photochemical activity was noted (**I, III**). Depending on the ambient temperature level and the time of the year, naturally occurring freezing temperatures resulted in either a minor temporary decline in photochemical activity or in a more permanent decline (**I**). A permanent decline coincided with day and night average temperatures below freezing and occasional night temperatures down to -10°C. This decline appeared earlier in populations of northern origins than in those of southern origins. The results are in accordance with earlier observations in spruce (Bolhàr-Nordenkampf and Lechner 1988, Lundmark et al.1988). In late autumn, after the photochemical decline in populations of northern origin, those of southern origins were able to respond to periods with relatively high temperatures (**III**). Possibly, this ability may be associated with the changed relative leader shoot growth pattern observed among clones when warm periods in autumn were followed with sudden drops in autumn minimum temperatures (**II**).

Apical mitotic activity in autumn,

as expressed by MI, ceased earlier and declined more sharply in populations of northern origins than in populations of southern origin (**IV**). Cessation of diameter growth showed no relation to the duration and level of apical MI (**II**). Among the studied clones no consistent difference in mitotic index (MI), either in period or in general levels was observed (**II**). Cessation of apical mitotic activity occurred later in selected populations than in natural populations of similar latitudinal origin (**IV**), indicating there was either an effect of plus-tree selection or a long-lasting effect of the seed orchard environment (Johnsen 1989a, b) on the timing of growth cessation. Cessation of mitotic activity appeared to be influenced by population origin, whereas the effect of temperature was unclear as the response of mitotic activity to temperature differed in spring and autumn. The results are consistent with results presented by Cannell & Willett (1975) showing that the point at which apical growth slowed down in autumn was closely correlated with latitude of seed origin. According to results from climate chamber experiments with detached shoots of *Picea abies*, cessation of apical mitotic activity appeared to be mainly influenced by day length (Fig. 5) (unpublished data).

Variation in cessation of apical mitotic activity in autumn, i.e. the duration of apical mitotic activity, appeared to be unrelated to the number of stem-units (NSU) produced in either lateral or leader shoots the following year (**II, IV**). Generally, NSU appeared to be

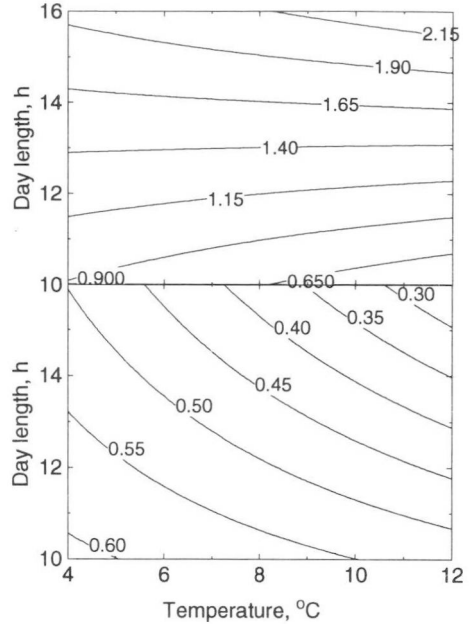Fig 5. Contour plots of the response of Mitotic Index (MI), %, (upper panel) and the Fv/Fm-ratio after freezing to -25°C, (lower panel) in lateral shoots exposed to different temperature and daylength conditions. Shoots were collected from non-juvenile trees (average latitudinal origin 63.8°N) in early August 1998 and subdivided into four equal groups (n=8). MI and Fv/Fm-ratios were determined using the same shoots, on Julian day 258 (Sept. 14) 1998, after five weeks in four different controlled environments: 10 h day/ 4 °C, 10 h day/ 12 °C, 16 h day/ 4 °C, 16 h day/ 12 °C.

correlated with tree height, leader shoot length and summer temperatures, whereas elongation of stem-units was mainly influenced by summer temperature. The accumulated height growth among natural populations showed poor growth performances in natural populations transferred more than approximately 3° in

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latitude, mainly due to lower number of stem-units (**IV**). In the study, southern populations did not show a lower ability to produce NSU than other populations. The results therefore indicated that the observed differences in height growth between southern and more northern populations, evident already in 1990, were related to differences in climatic adaptation. An effect of selection was indicated as selected populations appeared to produce more NSU than natural populations. Furthermore, of the three clones studies, the one with the greatest height growth produced more NSU and showed greater elongation of its stem-units than the other two.

Altogether, the results on growth cessation in autumn indicated genetic variation in cessation of growth both among and within populations. Cessation of growth occurred later in populations of southern origins and than in those of northern origins, and a selection effect on cessation of growth was observed. The level of photochemical activity appeared to be influenced by temperature, and remained at high levels until low freezing temperatures occurred in late autumn. Generally, the level of mitotic activity in autumn was influenced by temperature, as both the production of NSU and MI levels appeared to decrease with decreasing temperature. However, the influence of temperature on cessation of mitotic activity *per se* appeared to be small.

Hardening

In autumn, the frost hardiness levels of needles, as expressed by the Fv/Fm-ratio after freezing, gradually increased from early September to late October. Northern populations hardened earlier than southern populations (**III**) but no selection effects were observed. Furthermore, a difference in frost hardiness levels was observed among clones of similar origins, as frost hardiness developed 1-2 weeks later in one of the three studied clones (**I**). The rate of hardening appeared to be most rapid when the daily mean temperature fell to near or below +5°C (**I**) but was not related to the occurrence of frost (**III**). Northern populations were more frost resistant than southern populations, indicating there was a response to photoperiod. According to results from climate chamber experiments with detached shoots of *Picea abies*, needle frost hardiness, as expressed by the Fv/Fm-ratio after freezing to –25 °C appeared to be influenced by both day length and temperature (Fig. 5) (unpublished data). Results are consistent with those of Heide (1974) and Aronsson (1975), suggesting that both short-days and chilling temperatures are needed to induce cold hardiness in spruce. Also, Repo (1992) showed that, for both pine and spruce, temperatures between +10° to 0°C are the most efficient for inducing hardening.

In addition to the observed influence of temperature and photoperiod on the development of needle frost hardiness, the nutritional status of the needles appeared to affect hardening. Fertilisation appeared to promote the development of needle frost hardiness in autumn, as indicated by the results from a field study (Fig. 6) (unpub-

lished data). Furthermore, two of the three studied clones showed significantly lower levels of K and earlier hardening than the third clone (Clone 71) (**I** and unpublished data). The results are consistent with results in *Picea rubra* by Perkins & Adams (1995) and in *Picea sitchensis* by Jalkanen et al. (1998), where the timing of hardening and the level of midwinter hardiness were strongly influenced by deprivation of K.
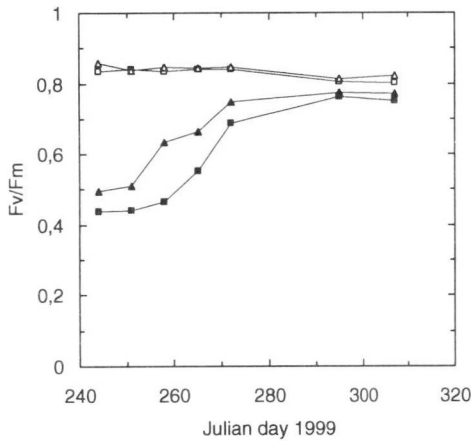

Fig. 6. Average Fv/Fm-ratios before (open symbols) and after (filled symbols) freezing to five temperatures (-5, -10, -17, -27 and -40 ºC) on each of seven occasions during autumn 1999. Current year needles were collected at 1-2 m height from non-juvenile trees of local origin at Flakaliden research area north of Umeå, treated with irrigation and liquid fertilization (Δ, $\blacktriangle$) (control, $\square$, $\blacksquare$). Samples were collected from four trees on each of two plots representing the different treatments. n= 8 samples × 5 temp. = 40.

Buds appeared to be less hardy than needles in early autumn, and de-

veloped hardiness approximately two weeks later than needles (**III**). In late autumn the hardiness levels appeared to be similar in buds and needles. Sudden drops in autumn minimum temperatures to below c. -12°C (-12± 1°C), following several weeks with mean temperatures above 3°C, affected subsequent shoot growth (**II**). The results are consistent with those of Beuker et al. (1998) who reported much higher rates of hardening in needles than in buds. However, in their study, needles hardened to higher frost hardiness levels than buds. Altogether, the results on the development of frost hardiness in needles and buds, indicated that needle frost hardiness is influenced by several environmental factors, and that bud frost hardiness appeared to be related to the mitotic activity within the bud. Therefore, it is possible that the observed variation in growth among natural populations was related to variation in frost hardiness levels among the populations.

In one-year-old seedlings of Norway spruce, strong correlations appeared to exist, at the population level, among growth cessation traits and between these traits and frost hardiness levels (**V**). The results indicated that timing of growth cessation traits e.g. bud set, height growth cessation, the degree of shoot lignification or frost hardiness levels could be used independently as indicators of growth cessation and frost hardiness level, at least at the population level (**V**). A clinal variation in cessation of growth was also observed, as latitude of origin explained a large part of the statistical

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variation among stands for the ana-
lysed traits, and growth cessation
tended to start later in populations of
southern origins than in populations of
northern origins. Effects of selection
on growth cessation and frost hardi-
ness level were observed. On average,
the seed-orchard progeny (selected
populations) performed similarly to
progeny from natural stands located 1-
2 degrees south of the origin of the
seed-orchard clones. Strong correla-
tions among timing of budset, shoot
lignification and frost hardiness level
in first-year seedlings at the popula-
tion level have also been reported in
other studies on Norway spruce (Pulk-
kinen 1993, Johnsen & Apeland 1988,
Skrøppa 1991). However, Skrøppa
(1991) and Johnsen & Apeland (1988)
found that the relationship between the
frost hardiness level and the timing of
budset was much weaker among fami-
lies than among populations.

Conclusions regarding autumn events

Altogether, the results on growth
and frost hardiness development in
autumn indicated genetic variation in
cessation of growth and frost hardi-
ness development, both among popu-
lations and among clones of similar
origin. Generally, cessation of growth
and frost hardiness development
tended to start later in populations of
southern origins than in populations of
northern origins. Variation in photo-
chemical activity before and after
freezing appeared not to be entirely
related to variation in levels of mitotic
activity either among populations or
among clones of similar origin. In non-

juvenile trees both the level of mitotic
activity and the level of frost hardiness
in autumn appeared to be influenced
by temperature. However, the level of
frost hardiness in needles appeared to
be affected by both temperature and
day length, whereas cessation of
growth in buds appeared to be mostly
affected by day length. Prolonged MI
activity in autumn may be associated
with a higher risk of frost damage to
buds. In contrast, in juvenile seedlings
strong correlations appeared to exist,
at the population level, among growth
cessation traits and frost hardiness
levels. The different shoot growth
patterns in juvenile seedlings and in
non-juvenile trees may affect the in-
terpretation of needle hardiness.

Validity of results and potential sources of error

The results are mainly based on
two field-test, at one location in north-
ern Sweden. Therefore, the results
must be interpreted with caution. They
may reflect specific conditions at the
location that are not generally applica-
ble, or conditions that contrast with
other test environments, such as those
prevailing at the field tests used to
determine "transfer rules" for planting
Picea abies within Sweden (Rosvall &
Ericsson 1981). Generally, these field
trials were established in slopes,
whereas this study was performed on
flat abandoned farmland.

In the clonal field-test (**I, II**) three
clones were selected to represent the
extreme range in accumulated height
growth among a population of clones

(rooted cuttings), selected through several previous selection steps. The clones studied may not, therefore, constitute a representative sample of either the population of clones studied, or of natural populations of similar origins. However, they do at least contribute to our general knowledge of seasonal rhythms of growth and hardiness development.

In the field-test with seedlings (**III, IV**), at least three potential effects of both natural and artificial selection may be present. Firstly, the high mortality in the field test may have led to unwanted selection, but if so the effect of this selection appeared to be non-systematic. Secondly, the sampling of trees for the study appeared to be slightly skewed, as the northern natural populations, compared with northern selected populations, were relatively taller in the studied sample than when all trees in the test were considered. The effect of this is probably negligible when MI and frost hardiness levels are concerned, but it may cause an underestimate of the growth performance of selected population. Thirdly, the study was not optimally designed with respect to the main methods of subsequent data analysis e.g. with populations grouped into southern and northern populations. Considering this, population limits should have been established first, and study trees should then have been randomly selected from all the trees in each population group.

Freeze tests of detached shoots (**I, III**) involve a higher degree of super-cooling, and lower freezing point, than those with intact shoots (Sakai & Larcher 1987). Similarly, freeze tests of needles or buds probably involve a higher degree of supercooling and a lower freezing point than those of other plant parts. Furthermore, ice-nucleation was not promoted artificially by, for example, spraying the needle samples with water before freezing. However, on other occasions when ice-nucleation was promoted by spraying needles with water or by adding nucleation powder, no effects on the relative frost hardiness levels were observed. The approach adopted is likely to have led to an overestimation of frost hardiness levels in all populations, but this would not have affected relative frost hardiness levels.

Mitotic activity was regarded as a discrete random variable with a binomial distribution, and individual cells as statistically independent trials (**II, IV**). The assumption that the individual cells were statistically independent trials may not be fully correct as it assumes a random distribution of cells in mitosis on the cell squashes. Based on the data obtained, however, slightly more mitoses occur at the edge of the cell squashes, and occasionally the distribution of mitoses appeared to be clustered, indicating that the individual cells may not be independent trails. Thus, the monitoring approach chosen affected the error and significance levels. However, it should not have affected the problem of decreasing variation that accompanies low MI figures, e.g. in early spring and late autumn.

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Concluding remarks

The results indicated genetic variation in initiation and cessation of growth, as well as in initiation of dehardening and hardening, both among populations and among clones of similar origins.

Natural populations of southern origins tended to initiate growth and dehardening later in spring and to start growth cessation and hardening later in autumn, than populations of northern origins. Natural populations transferred more than approximately 3° in latitude showed poor growth performances mainly because they had lower numbers stem-units. Based on these findings, the prolonged mitotic activity observed in southern populations seems to be of ambiguous value, since it may increase the risk of frost-related bud injuries rather than increase the amount of stem-units produced.

Progenies of selected plus-trees showed a later start of growth and slightly later dehardening in spring. Growth cessation occurred later in juvenile seedlings of selected populations. Furthermore, in non-juvenile trees of selected populations MI activity was prolonged, compared with natural populations of similar origin. Needle frost hardiness levels in selected populations were similar to those of natural populations of similar origin. Selected populations of northern origins tended to produce more stem-units than natural populations of similar origin. Growth and hardiness performances of southern populations and of selected populations of local origin appeared, at least in part, to have a similar physiological basis.

In spring, initiation of growth and dehardening appeared to be correlated with the later occurring bud burst both among populations and among clones, indicating that the present use of bud burst is valid and practical indicator of growth initiation. Initiation of growth and dehardening could not be solely explained by temperature.

In autumn, juvenile traits on juvenile seedling such as bud set, height growth cessation, degree of shoot lignification or frost hardiness level appeared to be possible indicators of growth cessation, at least at the population level. In non-juvenile populations, the amount of shoot growth appeared not to be correlated with duration of mitotic activity. Therefore, in non-juvenile populations, traits related to cessation of shoot growth appear to have less potential for use as indicators of growth cessation. However, it may be possible to use traits related to the duration of apical mitotic activity, e.g. bud frost hardiness, as prolonged mitotic activity could imply increased risk of bud injuries in late autumn. Initiation of growth cessation appeared to be influenced by photoperiod, and needle hardening by photoperiod and temperature. Generally, the development of needle hardiness in autumn appeared not to be fully correlated to levels of mitotic activity either among populations or among clones of similar origins.

Practical implications

•   The effects of the test environment on the effects of seed transfer and selection of individual trees need to be studied. For example, prolonged mitotic activity may represent a growth potential that is mainly expressed as high numbers of stem units in favourable environments, e.g. on south facing slopes, in contrast to less favourable environments, such as flat abandoned farmland, where it may lead to low numbers of stem-units.

•   Breeding of Norway spruce may benefit from more distinctly defined growth traits than those traditionally used, like accumulated height growth. For instance, it may be useful to separate growth traits related to growth rhythm, e.g. duration of mitotic activity, from traits which are not, or only moderately, related to growth rhythm.

•   To improve early testing, genetic correlations between similar traits in juvenile and non-juvenile trees must be identified. For example, the correlations between traits observed in juvenile seedlings, e.g. bud set and frost hardiness, with growth traits found in non-juvenile trees that are related to apical mitotic activity e.g. duration of such activity.

•   The internal factors and regulating substances involved in the seasonal development of growth and frost hardiness levels need to be characterised more fully, as the seasonal development of growth and frost hardiness levels is important for high growth performance.

•   The relationship between rate of cell division and production of stem-units needs to be studied, as high growth performance and high numbers of stem-units appeared not to be correlated with prolonged mitotic activity. New tools in cell molecular biology are now available that makes it possible to measure the rate of cell division, which should help in this task.

•   The relationship between needle and bud hardiness levels in non-juvenile trees and mitotic activity, e.g. duration of apical mitotic activity also needs to be investigated. This includes development of new methods for non-destructive bud hardiness measurements.

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