Winter Freezing Injury and Frost Acclimation in Planted Coniferous Seedlings
Winter Freezing Injury and Frost Acclimation in Planted Coniferous Seedlings
A literature review and case study from northeastern British Columbia

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PREFACE

The occurrence of winter damage to young conifer seedlings should be a concern to all silviculturists practising in areas where the problem exists. Similarly, the physics of the injury mechanism and the physiology of plant responses to winter climatic stress should be of interest to northern plant scientists. This report is aimed at both audiences in the hope that it provides a structured review of existing research and extension of the knowledge of seedling/environment interactions.

We hope the degree of detail, scientific expression, and practical significance of the report will suit both audiences. Silviculturists should appreciate the complexity of the mechanism of climatic adaptation by northern conifers. For them, the synthesis and summary of pertinent scientific information, concepts, and conflicting evidence should reinforce appreciation of the difficulty in providing a simple, practical solution to the winter injury problem.

For the scientist, we hope this brief review of a cross-section of research doctrine surrounding the issue of cold stress adaptation will be a convenient reference. Attempts have been made to include a wide range of references to pertinent original works to enable the reader to access greater detail as required. Discussion has been built around the practical application of research towards the improvement of northern coniferous forest establishment. Lastly, by indicating the direction being taken by the B.C. Ministry of Forests’ Research Program, we hope that greater collaboration between researchers and research agencies will speed resolution of this winter injury problem.

“No excuse is needed in this country for the study of the effect of frost upon forest trees. Owing to the very nature of the climate there is, perhaps, no commoner form of injury in the forest, and especially in young plantations, than frost damage. But perhaps, also because of the frequency of its occurrence, there is no form of damage which has been more frequently overlooked in the past or of which it is more difficult truly to estimate the importance.

“The diagnosis of diseases of this type is thus apt to present a problem which, though it may appear on the surface to be simple, is in reality rather complex, and one moreover which can only be unravelled by the most careful attention to detail both in the laboratory and in the field.

“As far as frost damage is concerned it is necessary to know what type of injury frost causes to the particular tree under observation, what the seasonal cycle of development of the tree is, what is the relation between this and the climate of the locality in which the tree grows, and finally what is the degree of frost at any particular season of the year at which the tree is liable to be injured.

“Moreover, experience shows that the careful collection of meteorological and phenological data in such a form as to be scientifically useful is a matter not only requiring care and experience but also involving considerable expense.”

— Excerpts from Day and Peace, 1934
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1 INTRODUCTION

In the boreal forest of northeastern British Columbia, significant losses of young, planted white spruce seedlings have been observed. The greatest toll is taken between the fall and spring of the first year after planting, but little damage occurs in successive years.

Silvicultural trials at Stewart Lake, near Dawson Creek, in the Boreal White and Black Spruce (BWBS) biogeoclimatic zone (Meidinger and Pojar 1991) have reported excessively heavy seedling damage (Herring and Letchford 1987). Each year over an 8-year observation period, newly planted white spruce seedlings have sustained injuries to foliage and stem tissues during their first winter in the field. This damage is abnormal since native spruces and older plantations have not exhibited serious injury during the same period.

Interviews with numerous silviculturists over several years indicate that observations made at Stewart Lake are not unique. Similar winter injury symptoms have been reported throughout the BWBS moist warm subzone (BWBSmw1) and wet cool subzone (BWBSwc1 and 2) from Quality Creek (latitude 55° 14'N) to Pink Mountain (latitude 57°N).

Figure 1 illustrates the area affected by the phenomenon. No symptoms of injury have been observed north of the Prophet River at approximately the boundary between the BWBSmw1 and BWBSmw2 subzones.

FIGURE 1. Area affected by seedling winter injury (hatched portion of the map) in the boreal forests of British Columbia.
Silviculturists practising within this area estimate that between 15 and 30% of their spruce plantations are visibly affected by first-winter seedling injury. During 1987, a total of 5.85 million 1- and 2-year-old container-grown spruce seedlings was planted within Dawson Creek and Fort St. John forest districts (B.C. Ministry of Forests, 1987).

There is reason to suspect the incidence of damage may be somewhat higher than reported since survival inspections are not usually carried out until sometime during the second growing season or later. This makes it difficult to assign mortality to the season of occurrence and, after a season of recovery, symptoms of winter injury may be mistakenly attributed to other causes.

From evidence gathered at the Stewart Lake study site, it appears that winter climate, or winter climate-related phenomena, may be implicated in the damage to young spruce seedlings. It follows that the subfreezing temperatures that characterize winters in the boreal forest may be a direct or indirect cause of injury.

First-winter seedling injury could potentially limit the successful establishment of white spruce in the boreal forests of British Columbia. The phenomenon may adversely affect plantation establishment over a large geographic area by decreasing seedling growth and increasing seedling mortality. Furthermore, the early growth performance of even visibly uninjured seedlings may be affected after the first winter period.

Providing a solution to the winter injury phenomenon in northeastern British Columbia requires study. The response of plants to freezing temperatures is complex and involves various processes at the cellular level that must first be understood before the cause of the problem can be determined. Also, to understand why white spruce seedlings are damaged during their first winter while older seedlings and established natural regeneration are not, the type of injury and the primary cause must be clearly identified.

According to Roberts and Miska (1980), about 3000 scientific publications pertaining to winter freezing injury and frost hardiness were produced between 1965 and 1975. Such an abundance of literature indicates the importance of this field of research and the enormous interest the problem has generated among scientists. If one considers the studies published before 1965 and after 1975, it is obvious that keeping abreast of current information is a major task.

The published information on the subject has not been summarized or evaluated (Steponkus 1984). Instead, a number of “schools of thought” have emerged and their representatives advocate their own views—a situation that does not help improve our understanding of frost hardiness and frost injury related phenomena.

1.1 Report Objective

The purpose of this report is to provide forest managers with a better understanding of the winter injury phenomena, and to explain how plants are killed or damaged, and how frost hardiness is acquired and lost. Observations from an 8-year-old study and from current studies of seedling injury in northeastern British Columbia will serve as a background to the discussion of possible causes of the injuries.

The report first discusses the physiological processes, at the cellular and whole plant levels, that enable boreal plant species to survive the stresses of winter. Next, the form of injury sustained by planted spruce seedlings is explored in detail. Finally, the report identifies the possible damaging agents involved in the observed injuries and discusses potential preventative measures.

This report marks a renewed interest in cold stress physiology and in the study of winter injury to seedlings in northeastern British Columbia. A number of new studies that address the problem are currently under way at Red Rock Research Station (B.C. Ministry of Forests) in Prince George.
2 THE FREEZING PROCESS

Low winter temperatures in northern latitudes result in severe stresses to native plants, stresses that are related to the freezing of plant tissues. The process of freezing in plant tissues is complex, involving the redistribution of water both in its physical state and location. The redistribution depends on rates of cooling and warming, as well as on the type of tissue involved (Steponkus 1984).

The rate of cooling is considered slow if it proceeds at 1–2°C/h (for an entire plant) and rapid at 5–20°C/h.

In nature, the rates of cooling and freezing are usually slow, often less than 1°C per hour (Levitt 1972, 1980). The freezing process was first described by Scarth and Levitt (1937) and Siminovitch and Scarth (1938), but the detailed physico-chemical analysis of this process was presented by Mazur (1969, 1970), and became the cornerstone of cryobiology.

When the temperature slowly falls to below 0°C, plant cells and extracellular solutions remain temporarily unfrozen, in a state referred to as “supercooled.” Eventually, ice crystals form in the extracellular solution at a temperature that depends on the solution’s freezing point and the presence of ice nucleating agents. As a result of ice formation outside the cell, the solute concentration of the unfrozen portion of the extracellular solution increases and, consequently, its osmotic potential becomes more negative. The freezing process continues until the chemical potential of the ice and that of the remaining unfrozen solution reach an equilibrium.

This process is complicated. It involves the spatial distribution of the solute concentrations in relation to the ice interface and depends on the time and velocity of ice growth (Körber and Scheiwe 1983).

The increasing solute concentration of the extracellular solution has an effect on the intracellular solute concentration. The chemical potentials of both extra and intracellular solutions must also reach an equilibrium by either intracellular ice formation or cell dehydration. At slow rates of freezing, water migrates from the cell to the sites of extracellular ice formation at a rate dependent on the water permeability of the cell membrane (plasma membrane) and the surface area to volume ratio of the cell (Steponkus 1984).

It is the stability of the plasma membrane that determines the rate of water migration from the cell, and whether the equilibrium between the extracellular and intracellular solution potentials is reached by dehydration or the formation of intracellular ice (Steponkus 1984). If the rate of cooling is rapid, water efflux from the cell is insufficient and supercooling of the intracellular solution may occur. However, the formation of intracellular ice requires either the presence of ice nucleating agents, which are normally not present in the protoplast (Mazur 1977), or seeding by external ice. The plasma membrane is an effective barrier to extracellular ice (Mazur 1977) and seeding by external ice would occur only if the plasma membrane were damaged or penetrated by ice (Steponkus 1984).

Spontaneous nucleation (without nucleators) of pure water occurs at -39°C, and at lower temperatures in the concentrated solutions of the cell protoplast (Rasmussen and MacKenzie 1972). Inoculation by external ice could occur during rapid cooling to a sufficiently low temperature that ice crystals would be small enough to penetrate the plasma membrane (Mazur 1963).

It is widely accepted that intracellular freezing is fatal for the tissues of all plants (Tumanov and Krasavtsev 1959; Glerum 1985). However, extracellular freezing also places severe stresses on plant tissues. The detrimental effects of the freezing process on plant function is discussed in more detail in Section 4, “Frost-related Injuries to Plant Tissues.”

---

1 There is a difference between rates of cooling and rates of freezing. The former refers to temperature changes in the medium surrounding the freezing object (e.g., air temperature around a plant). Rates of freezing refer to the freezing process itself (see definition of freezing in the text). Since, in most cases, cooling rather than freezing rates are measured, this term will be used throughout this text.

2 Note: the meaning of words marked by an asterisk and italicized is explained in an alphabetical glossary at the end of the report.
3 PLANT ADAPTATIONS FOR LOW TEMPERATURE SURVIVAL

Some plants are able to survive low temperatures better than others. Levitt (1980) describes two basic types of mechanisms that enable a plant to survive subfreezing temperatures: freezing avoidance and freezing tolerance. He points out that while plants do not tolerate intracellular freezing, they (or their parts) are often tolerant of extracellular freezing. The tolerance is usually seasonal and in perennial herbaceous plants it is linked to reduced growth activity (Gusta and Fowler 1979; Sikorska and Kaperska-Palacz 1979), and in woody plants of temperate regions it is linked to dormancy (Tumanov 1967; Weiser 1970). Processes such as water relocation, supercooling, decreasing of the freezing point of cellular solution, and tissue desiccation enable the plant to avoid intracellular or even extracellular freezing.

3.1 Strategies for Freezing Avoidance

Freezing avoidance applies to entire plants or to tissues and organs of partially freeze-tolerant plants (Sakai and Larcher 1987). It is a mechanism by which the plant excludes the harmful effects of even extracellular freezing from its tissues. This is usually achieved by the migration of water from these tissues and deep supercooling of the intracellular solution. Four freezing-avoidance strategies are briefly described here.

3.1.1 Supercooling

Plant tissues are temporarily able to remain unfrozen at sub-zero temperatures through the process of supercooling. Supercooling is possible in the absence of ice nucleators that would otherwise initiate freezing (Fitter and Hay 1987). The supercooled state is usually transient and unstable. It only provides protection against brief frosts from 3 to 8°C below the freezing point of tissues (Hatakeya 1960, 1961; Kaku 1975; Marcellos and Single 1979). In acclimatized tissues, however, deep supercooling may occur to about -39°C (the temperature of spontaneous nucleation of water [George and Burke 1977]). In species that can persistently supercool, plant tissues may remain supercooled for longer periods of time. Tissues such as xylem ray parenchyma, which have almost no space for extracellular ice formation, may depend entirely on supercooling (Sakai and Larcher 1987). Floral and vegetative buds of a variety of species exhibit persistent supercooling. Cell dehydration does not have to occur during true supercooling (Sakai and Larcher 1987).

3.1.2 Changes in cell solution concentration

Changes in cell chemistry may result in solute concentration changes in the intracellular solution, effectively depressing its freezing point. The simplest example would be conversion of starch to soluble sugars during the acclimation period (Sakai 1966). It should be noted that cell sugars do not only passively protect against freezing by elevating cell solute concentration, but also directly protect cellular membranes (see Steponkus [1984] for discussion).

3.1.3 Tissue desiccation

Minimizing water content is the principal way seeds survive harsh conditions (Sakai and Larcher 1987). However, low water content has also been implicated in increased frost survival of buds, leaves, and stems. The mechanism is based on the removal of freezable water from cells (Sakai and Larcher 1987). Desiccation by water migration from cells to extracellular ice, as well as from tissues and organs to other sites of ice formation (see Section 3.1.4), may occur in conjunction with supercooling and increased solute concentration. Organs and tissues of different plants vary in their capability to withstand desiccation and this may delimit their survival (Sakai and Larcher 1987).
3.1.4 Extraorgan and extratissue freezing

When water is translocated from supercooled tissues and organs to ice nucleation centres, extra-
tissue and extraorgan freezing occur (Sakai 1979). This type of protection against freezing has been
reported for a variety of plants, tissues, and organs, including hydrated seeds (Ishikawa and Sakai 1982),
floral primordia of woody angiosperms where ice nucleation centres are located in bud scales (Dorsey
1934; Graham and Mullin 1976; Quamme 1978), and the vegetative buds of conifers (Alden 1971; Sakai
1979, 1982).

An interesting example of extraorgan freezing is demonstrated in the freeze-avoidance of bud tissues
in certain conifers. In the Abietoioideae and Laricoideae, ice forms primarily in the pith beneath the so-called
crown tissue. This is believed to result from less supercooling in the pith than in the primordial shoot (Alden
1971; Sakai 1979, 1982). Primordial shoots are also thought to lack ice nucleators (Alden 1971), which
reduces the likelihood of ice formation.

3.2 Strategies for Freezing Tolerance

The ability to survive subfreezing temperatures, referred to subsequently as frost hardiness, depends
largely on the physiological state of the tissue and plant. Active growth must cease before the process of frost
hardiness can develop (Glerum 1985). Plants tolerate freezing by becoming dormant and frost hardy. It must
be emphasized that dormancy development and frost hardening are coincidental but separate processes.
There are several types of dormancy and all may occur without frost hardening. Usually, however, frost
hardening is associated with dormancy development.

In contrast to freezing avoidance, freeze-tolerant plants (or tissues) can, seasonally, withstand the
formation of extracellular ice. The formation of ice crystals is confined to spaces outside the living cell
(intercellular spaces) (Sakai and Larcher 1987). Many of the hardy deciduous species easily withstand
extracellular freezing in the xylem (Sakai and Larcher 1987).

3.2.1 Plant dormancy

By definition, living tissue is dormant if, while exposed to growth-promoting conditions, it does not
elongate or grow in some other manner (Molish 1922; Doorenbos 1953). There are different types of
dormancy, but in this report only the winter-related period of growth inactivity is discussed. Various terms
have been used for this type of dormancy, including “winter dormancy,” “true dormancy,” “rest,” and
“winter quiescence” (see Lang et al. 1985).

Unfortunately, dormancy has not been easily or accurately defined (Romberger 1963; Champagnat
1983; Lavender 1991). In woody plants, particularly conifer seedlings, bud dormancy is of primary concern
as it relates to the end of shoot elongation. In many physiological studies, bud dormancy is considered
to be a period of time between the so-called budset (bud scales can be easily discerned) and bud flushing
(visible effect of shoot elongation) (Lavender and Hermann 1970; van den Driessche 1975, 1977;
Lavender 1981). The ability of inactive buds to resume growth when placed under favourable conditions
differs throughout the fall to spring period. For this reason, additional subdivisions of the dormant period
were proposed (Vegis 1964; Perry 1971). A distinction between dormancy and quiescence has been
suggested: dormancy relates to the lack of growth resulting from endogenous control; quiescence results
only from environmental conditions unfavourable to growth and, once these become favourable, growth
will resume (Champagnat 1989). Even these interpretations, however, are not without problems.
Anatomical studies of bud development in many conifers show that initiation of bud scales (or leaf
primordia in free growing seedlings) and axillary buds occurs well before bud flushing (Owens 1968). Also,
immmediately after budset, dormancy could be considered to be the deepest, since further shoot elongation
is usually not easily evoked during this period. Nevertheless, considerable growth activity occurs within
the bud at that time (Owens and Molder 1973) and mitotic activity of the shoot apex accelerates during
early initiation of leaf primordia (Cannell and Cahalan 1979).
Several authors' concept of dormancy (Perry and Simons 1967; Kozłowski 1971) permits some amount of bud enlargement or initiation of bud scales during dormancy. This latitude does not agree with the concept put forward by others of dormancy as a mechanism of survival during harsh environmental conditions (Vegis 1964; Lavender and Cleary 1974), since frost hardiness is lost rapidly during bud enlargement (Alden 1971).

Another approach considers different "depths" of dormancy (e.g., Fuchigami et al. 1982; Ritchie 1989). In this approach, days to budbreak indicate the depth of dormancy. The degree growth stage model (Fuchigami et al. 1982; Green and Fuchigami 1985) attempted to explain the relationship between developmental stages of terminal vegetative buds and the development and state of frost hardiness. This approach is useful to forest managers, and is used to determine the best times for lifting, storing, and planting nursery-grown stock.

The lack of a uniform understanding of dormancy is partially due to a confusing terminology. Attempts have been made, with little success, to introduce a uniform terminology pertaining to different kinds of dormancy, and to define their characteristics depending on the origin and location of controlling factors (e.g., Romberger 1963; Lang et al. 1985).

3.2.2 Frost hardiness

Frost hardiness in seedlings can be defined as the lowest sub-zero temperature to which a seedling can be exposed without damage (Glerum 1985). Acclimation (becoming frost hardy to suit winter climate) involves a number of biochemical and ultrastructural changes that enable a plant to withstand low temperatures.

Frost hardiness develops and deepens during the cessation of active growth. It is first triggered by decreasing day length (photoperiod) and then by low temperatures (Turmanov and Krasavtsev 1959; van den Driessche 1969; Weiser 1970, 1986). At least two (Turmanov and Krasavtsev 1959) and possibly three (Weiser 1970, 1986) stages of frost hardiness have been distinguished.

**Stage-one hardening**

The first stage of frost hardening (plants can withstand mild frost) is attained in native Canadian coniferous species as the photoperiod decreases during fairly warm days and cool nights (Glerum 1985). Weiser (1986) states that a plant's vegetative maturity (most growth processes for the current growing season are completed) is reached at this time and dormancy begins. He also suggests that plant dormancy is a hormone-induced response. Hormones are thought to be produced in leaves and are distributed throughout the plant. Weiser (1986) attributes stage-one frost hardening to the phytochrome enzyme-pigment system.

**Stage-two hardening**

The second stage of frost hardening is facilitated by low temperature (Sakai and Larcher 1987), and requires slight frosts to develop (Weiser 1970, 1986; Sikorska and Kacperska-Palacz 1979). Structural changes occur in plant cells, particularly the synthesis or modification of membrane lipids and proteins (Sikorska and Kacperska 1982; Sakai and Larcher 1987).

The accumulation of starch reserves and neutral lipids in the fall (Cecich 1977; Krasowski and Owens 1990) provides essential substrates and a source of energy for metabolic changes taking place at the second stage of frost hardening (Sakai and Larcher 1987). Changes in levels of storage products have been widely reported, particularly autumn increases in starch levels (during stage one), followed by reductions (during stage two) as further hardening occurs (Srivastava and O'Brien 1966; Pomeroy and Simonovitch 1971; Kupila-Ahvenniemi et al. 1978; Krasowski and Owens 1990). In vegetative buds of Scots pine, a decrease in starch at that time (stage two) is paralleled by an increase in stored tannins (phenolic compounds) and this relationship is reversed again at the resumption of growth in spring (Hejnowicz 1979).
During stage two of frost hardening, plant response is localized and, as expressed by Weiser (1986), "initiation of physiological rest begins." Progression from stage one to stage two is rapid (Weiser 1986). Some researchers (e.g., Weiser 1970, 1986) distinguish a third stage of frost hardiness involving spatial modifications of macromolecules. This stage is triggered by prolonged exposure to subfreezing temperatures and is lost as soon as the plants are thawed.

Boreal conifers such as white spruce have adapted through genetic selection to survive very cold winters (Ying and Morgenstern 1982). By synchronizing their phenology to annual climatic cycles, they attain safe levels of hardiness over all seasons. They react to environmental cues of changing photoperiod and temperature to make appropriate adjustments in cell structure, chemistry, and physiology.

The combination of inherited adaptation and regional climate is a provenance-specific effect that determines periods of active growth and dormancy. Ekberg et al. (1985) observed this growth rhythm in Norway spruce as reflected in the timing of bud flush and budset. Levitt (1980) emphasizes the importance of a "seasonal rhythm" to winter hardiness. Cannell and Sheppard (1982) observed six distinct phases in the rhythm of cold hardiness of Sitka spruce in Scotland. Jonsson et al. (1986) indicated the importance of the autumn hardening phase to lodgepole pine for protection against autumn frosts. The tree must be ready to respond, on cue, to photoperiodic and thermoperiodic changes in order to tolerate extracellular freezing or avoid injury in some other way.

Acclimation to low temperatures and the development of frost hardiness is a time-dependent process. Campbell and Sorensen (1973) found that the greater the period between budset in Douglas-fir and the occurrence of fall frosts, the greater the resistance to cold injury. Colombo (1984a) observed that black spruce seedling winter injury was greater for specimens with the greatest shoot extension, and presumably the latest budset. Jonsson et al. (1986) suggest that delayed autumn hardening may affect the stability of hardiness during late winter temperature fluctuations.

Green and Fuchigami (1985) identify late winter as a critical time for winter hardiness. They indicate that only very low temperatures at this time will maintain winter acclimation, while higher temperatures will initiate deacclimation. For instance, once dehardening is initiated, lodgepole pine exhibits a much lower tolerance to recurrent low temperatures (Jonsson et al. 1986).

Different tissues within the same plant exhibit different relative levels of frost hardiness that change seasonally (Alden 1971; Glerum 1985). For instance, the aboveground portion of the plant is much more hardy than the roots (Stepankus et al. 1976). Also, Glerum and Farrar (1966) showed that xylem of spruce was most sensitive to freezing during cell differentiation, whereas cambium and, particularly, phloem were less sensitive. Day and Barrett (1963) found that needles of Pinus sylvestris were more susceptible to freezing injury than other parts of the plant in autumn. In white spruce, however, buds and cambium were more susceptible to frost damage in autumn than were needles (Hawkins and Draper 1991). Lateral bud development was 2–3 weeks slower than that of terminal buds in mature Douglas-fir (Owens 1968), and until late fall they were also less frost hardy. In winter, however, lateral buds were more hardened than terminals. It is possible this relationship is reversed in seedlings (Alden 1971).

Owens (1968) reported that terminal buds of mature trees lost much of their hardiness in February, whereas other tissues exhibited a higher level of frost hardiness.

Belowground, although roots do not acquire dormancy (Green and Fuchigami 1985; Dormling 1986), their frost hardiness changes seasonally (Mityga and Lanphear 1971; Glerum 1985; Smit-Spinks et al. 1985). Since roots are protected from drastic temperature changes by soil, their hardiness is lower than that of shoots and more readily altered by changes in temperature of the surrounding soil (Glerum 1985).
Root hardiness is primarily a function of soil temperature (Green and Fuchigami 1985). Low soil temperatures are needed by roots to become frost hardy. Root hardiness develops gradually prior to soil freezing (Glerum 1985, citing Timmis, pers. comm.) and is lost earlier than occurs in aboveground tissues (Glerum 1985). Roots may completely deacclimate within 24 hours if subjected to warm temperatures, and require many days to re-harden when temperatures fall.

The depth of the root system is also important in the frost hardiness of roots. Shallow-rooted species such as spruce require roots that are more frost hardy than deeply rooted species (Lindström 1987). Lindström (1987) reports that the moisture content of roots decreases during hardening, in a manner similar to that in shoots.

4 FROST-RELATED INJURIES TO PLANT TISSUES

4.1 Seedling Injuries in the Peace River Region

The actual cause of spruce seedling injuries in northeastern British Columbia is difficult to ascribe because of the wide range of factors potentially involved before, during, and following planting. The observed normal winter survival of white spruce natural regeneration and that of older planted seedlings further confuses attempts to identify a single cause.

The symptoms of 1st-year winter injury at Stewart Lake have been observed on all parts of newly planted spruce seedlings, including buds, needles, and stems. Symptoms range from slight damage to seedling death. Slight damage is usually limited to needle tissues, where browning of nursery or 1st-year foliage is the usual symptom. There is no visible injury to shoots or buds, which flush normally during the following spring. Damage of this type is limited to the upper portion of a seedling, and is often concentrated on its southwestern side. Figure A1, Appendix A shows various degrees of injury to planted spruce seedlings.

Bud damage usually accompanies severe foliage injury. Most often, terminal buds alone are damaged, whereas lateral buds may flush normally. It is unclear, however, whether bud loss is due to damage to the bud or to the stem immediately below the bud. Injury symptoms are often localized at one-half to three-quarters of the seedling's height, in the area near fluctuating snow level.

The most severe symptom of seedling injury is the death of all or a large portion of the seedling shoot. Stem death results in needle and bud death on the portion of the stem killed; lower needles and shoots may survive with no apparent injury. Loss of either 1st-year shoots or both 1st-year and nursery shoots is common. Severe damage destroys all but the root collar area of the seedling, where repressed lateral shoots or buds are the only surviving aboveground portion of the seedling. At its worst, winter injury may result in the death of the entire seedling.

With only a few exceptions, seedlings that survive the first winter are usually not injured during subsequent winters.

Seedling injuries appear to take place during the winter months of November through March. Careful observations of seedlings on research sites reveal that winter injuries do not occur until November. Winter injury symptoms usually become evident during April, with the onset of warmer spring temperatures.

4.2 Hypotheses and Mechanisms of Freezing Injury

Steponkus (1984) suggested that the main problems limiting the understanding of cold-related injuries are twofold: insufficient information about the nature of damage at the cellular level, and the tendency for some researchers to confuse primary injury with secondary injury.

The term "freezing injury" has now replaced the previously used term "frost injury." This seemingly minor change in terminology is conceptually important. It recognizes the possibility that injury or death of cells, tissues, or the entire plant may not be caused directly by low temperature (frost), but that it may also result indirectly from other accompanying factors. This notion evokes an important question: does freezing stress always produce the same kind of injury, or might there be several types of injury?
4.2.1 A two-factor hypothesis of freezing injury

The oldest hypothesis on freezing injury emerged during the 17th century. It attributed injury to cell rupture by ice, and was based on observations that water increased its volume while freezing. This hypothesis was abolished with observations that cell volume actually decreases during freezing (reviewed by Levitt 1980).

More recently, Mazur (1969, 1970) presented a two-factor hypothesis on freezing injury. He suggested that water freezes inside the cells of plant tissues subjected to rapid rates of cooling. The resulting intracellular ice injures cell organelles and membranes. At slow rates of cooling, however, water migrates from the cell to external ice, and the resultant cellular dehydration effects are the source of other forms of injury. A similar concept was offered by Levitt (1972, 1980), who distinguished between direct and indirect freezing injuries. He attributed direct freezing injury only to intracellular ice formation, but indirect injuries to secondary stresses associated with the freezing process. While both concepts have received widespread acceptance, Steponkus (1984) warned that inferring a simple cause-and-effect relationship might be misleading.

4.2.2 Factors influencing the occurrence of freezing injury

Various factors influence the occurrence and severity of freezing injury, other than simply the phase change from water to ice. For instance, there is evidence of increased injury after rapid thawing (see Levitt [1972, 1980] for review), although rates of warming (thawing) have been found less critical for freezing injury than rates of cooling. Rapid thawing may be more destructive to non-acclimatized than acclimatized plants. This may be due to the mechanical failure of the plasma membrane during rapid re-hydration of the cell, because the plasma membrane is more prone to this type of injury in the non-acclimatized stage (Steponkus 1984).

It has been reported that repeated freezing and thawing increases the likelihood of freezing injury (Levitt 1980). For instance, repeated freeze-thaw cycles caused significant disturbances to the thylakoid membranes* of chloroplasts of Pinus silvestris (Martin and Qvist 1979). Venn (1979) found a significant increase in damage to Norway spruce with an increasing number of exposures to freezing conditions. This may be due to the stresses imposed on cellular membranes. Christersson and Sandstedt (1978) observed repeated and rapid fluctuations in temperature around the freezing point during late winter and spring, or during milder temperatures and intermittent cloudiness.

The length of time tissues are frozen is also an important factor determining degree of injury. For short periods of time, up to 24 hours, the duration of the frozen state is not crucial (Levitt 1956), but injury increases with longer durations (Sakai 1966). In nature, the problem of relating injury to length of time frozen is complicated by repeated thaw events (Levitt 1972, 1980).

Conditions after thawing influence the degree of recovery of damaged tissues. It is apparent that severe injuries cannot be repaired regardless of post-thawing conditions, but some incipient forms may be reversible (Levitt 1972, 1980). Recovery from incipient injury has been reported (Palfa et al. 1977, 1982) and is facilitated by low light conditions. Tissue recovery is apparently related to the recovery of the photosynthetic apparatus (Steffen and Palfa 1987).

4.2.3 How freezing injury is manifested at the cellular level

Injury observed at the whole plant level is a manifestation of injury to individual cells. Views of what constitutes injury at the cellular level are not uniform. Rates of cooling and physiological state of the plant (acclimatized or not) determine how the injury is manifested at the cellular level. According to recent views, the plasma membrane (the sack-like membrane that contains the living portion of the cell) plays a central role in freezing injury.

Steponkus (1984) published a review of the role of the plasma membrane in freezing injury. Highlights of his interpretation of the mechanisms and manifestations of freezing injury to the plasma membrane are presented here. Original references to the sources of information are cited, where appropriate.
Damage to the plasma membrane is the crucial determinant of injury to a cell and may be exhibited as follows:

**Expansion-induced lysis**

Extracellular freezing results in cell membrane contraction (*plasmolysis*) when water leaves the cell. Lysis (rupture) of the plasma membrane has been observed during deplasmolysis (reverse of plasmolysis), or the re-swelling of the cell during thawing as water returns to the protoplast. This is particularly noted after thawing of non-hardy tissues (Levitt and Scarth 1936; Siminovitch and Scarth 1938).

The incidence of injury during deplasmolysis is correlated with the degree of plasmolysis (Scarth et al. 1940; Siminovitch and Levitt 1941). In experiments with isolated protoplasts, it has been shown that cell survival is directly related to the magnitude of the change in plasma membrane surface area during expansion (deplasmolysis) (Wiest and Steponkus 1978). In non-acclimatized cells, contraction during freezing causes loss of membrane material (via *endocytic vesiculation*). Simply speaking, the plasma membrane during plasmolysis has too large a surface area, so it self-trims itself to fit the shrinking protoplasm. Williams and Hope (1981) suggested the loss of material from the plasma membrane is irreversible. Upon deplasmolysis, to avoid injury, the plasma membrane must withstand the pressure resulting from increasing cell volume. This can be achieved by incorporation of new membrane material to increase the surface area.

In non-acclimatized cells, the readily available membrane material is in an insufficient quantity to be reincorporated at a rate that would prevent rupture of the plasma membrane and, consequently, lysis occurs (Dowgert and Steponkus 1983). Expansion-induced lysis is probably the main form of freezing injury in non-acclimatized cells, though not the sole one (Steponkus 1984). The ability of the plasma membrane to expand without injury increases after cold acclimation (Steponkus et al. 1981).

**Loss of osmotic responsiveness**

This form of injury is related to plasmolysis as described by Levitt (1972). After contraction and plasmolysis, at slow rates of cooling, protoplasts become osmotically inactive and remain contracted even after thawing. This form of injury takes place during freezing at lethal temperatures (Rajeshkara et al. 1979).

Loss of osmotic responsiveness, rather than mechanical disruption of the plasma membrane, predominates in acclimatized tissues. The causes of altered osmotic behaviour of the plasma membrane are not known. However, changes in membrane structure at lethal temperatures have been reported (Singh 1979; Singh and Miller 1982). Several mechanisms of membrane destabilization have been hypothesized:

**Solute concentration effects** Most information on the effects of high solute concentrations on the stability of biological membranes is based on studies of chloroplast membranes (thylakoids) (Heber 1967, 1968; Heber and Santarius 1973; Steponkus et al. 1977; Steponkus 1979; Heber et al. 1981). Since effects on other cell membranes have received less study, these findings provide the basis for general interpretations of similar effects on any membrane, including the plasma membrane.

Heber et al. (1981) suggested that high solute concentrations of inorganic electrolytes and various organic compounds may cause dissociation of the cell membrane bilayer. This results in an altered charge distribution, the opening of *hydrophilic* channels, and, ultimately, membrane collapse (Jensen et al. 1981). This theory is questioned in some reports (Meryman 1968; Rajeshkara et al. 1979; Williams and Shaw 1980).

**Removal of water** Removal of water from the face of the membrane may result in changes in membrane configuration (Luzzatti 1968). The influence of water removal on membrane stability is not understood and requires further investigation. The interaction effects of water removal and high solute concentrations are suspected of influencing membrane stability (Steponkus 1984).
**Electrical perturbations**  Electrical perturbations can arise from temporary differences in the electrical potential between liquid and solid phases (Workman and Reynolds 1950), as well as from the generation of large ion concentration differentials across the membrane (Steponkus 1984, citing J. Wolfe, pers. comm.). As a result, solute permeability of the membrane may increase and structural failures may occur (Zimmermann et al. 1981; Zimmermann 1982).

**Thermotropic** phase transitions  This theory is the only one that implicates low temperatures alone as a cause of injury. It is therefore distinct from others, which implicate factors such as ice and dehydration as the primary damaging agent (see Levitt 1972, 1980).

It has been suggested that low temperatures cause phase transition changes of the cell membrane, by altering the arrangement of its lipo-protein constituents and thus changing the membrane fluidity (Yoshida 1982, 1984). Steponkus (1984) cautioned against subscribing to this theory because it is well known that plant tissues can withstand very low temperatures without injury in the absence of ice.

**Altered osmotic behaviour**

This is another form of injury observed in the isolated protoplast during warming. Osmotically responsive protoplasts do not regain their full volume after thawing, apparently due to a prior loss of intracellular solutes or a leakiness of the plasma membrane. Steponkus (1984) suggested that this type of injury may correspond to that reported by Palta and co-workers (Palta et al. 1977; Palta and Li 1978, 1980; Palta et al. 1982), in which active transport through the membrane (ATPase dependent) was apparently altered. This type of injury may be reversible.

**Intracellular ice formation**

Intracellular ice formation has been viewed as the only direct form of freezing injury that always results in cell death (Weiser 1970; Levitt 1972, 1980). An early, mechanistic explanation (Levitt and Scarth 1936) assumed that the limited permeability of the plasma membrane to water movement was the main cause of intracellular ice formation. Once formed, intracellular ice crystals are believed to disrupt the protoplasmic and organelle structure mechanically, as well as destroy cell membranes, resulting in the loss of their semipermeable properties (Levitt 1972).

Much controversy surrounds existing explanations of how intracellular ice forms, and its damaging effects on the cell. For instance, the major criticism of Levitt and Scarth's (1936) theory is its failure to consider the requirement for ice nucleation in the supercooled intracellular solution (Steponkus 1984). The authors also suggest that the water permeability of the plasma membrane changes seasonally and increases during cold acclimation of the plant. Only a few studies have supported this assumption (e.g., McKenzie et al. 1974), and some have contradicted it. Sukumaran and Weiser (1972) and Stout et al. (1977) either report no detectable changes in the permeability of the plasma membrane, or indicate that in nature the water permeability of the plasma membrane is sufficient for free water movement.

Mazur (1977) and Fujikawa (1980, 1981) suggested that physical contact between intracellular ice and the plasma membrane would result in injury. Steponkus (1984) indicated, however, that since extracellular ice is frequently in contact with the plasma membrane without causing obvious damage, there is little basis to presume that direct contact between intracellular ice and the plasma membrane should cause injury.

Siminovitch and Scarth (1938) considered that both intra- and extracellular ice formation would damage the plasma membrane. Others, however, believe that intracellular ice formation is not the cause but a consequence of injury to the plasma membrane (Steponkus and Dowgert 1981; Dowgert and Steponkus 1983).
The theory of intracellular ice formation and the central role of the plasma membrane in freezing injury remains widely accepted. However, the mechanisms involved have yet to be fully understood. A satisfactory explanation is confounded by the difficulty in observing intracellular ice formation, even with modern advanced techniques (Levitt 1972).

4.3 Desiccation

4.3.1 Evaporative freeze-desiccation

Freeze-desiccation is synonymous with "frost drought." Freeze-desiccation injury is attributed to the evaporative loss of water from the plant when translocation of water from soil or frozen parts of the plant (e.g., roots or xylem) is impossible. Levitt (1980) did not consider this type of injury of major importance in the severe climates of the northern USA and Canada, but suggested that it may be important in other climatic regimes. Sakai (1970) reported that freeze-desiccation injury was the most serious type of winter damage observed in eastern Hokkaido. This injury was most common on southern slopes where the temperature of sun-exposed leaves and stems could rise to 17°C at mid-day while the soil temperature remained below 0°C. Under such conditions, intense dehydration occurred by the end of February, which resulted in the browning of foliage.

Winter desiccation has been considered the greatest limiting factor to tree growth in Japan (Sakai 1968). Desiccation is intensified by sweeping winds and lack of snow cover (Sakai 1968, 1970). Under these conditions, damage may occur regardless of the slope aspect (Sakai 1968). Twigs and stems above the snow gradually dehydrate from the tip downwards and are usually killed by the end of winter. Sakai (1970) demonstrated that artificially induced desiccation of white spruce resulted in stem damage as well as needle death. In contrast to Levitt (1980), Sakai (1968) suspected that winter desiccation could be a major cause of seedling mortality and injury in mature trees in the northern USA and Canada. "Red belt" (browning of injured foliage on trees located at medium-high elevation), a well-known phenomenon occurring in the Rocky Mountains, is suspected to be the result of winter desiccation (Sakai 1970). The physiological explanation for red belt remains inconclusive.

Winter desiccation injury may be related to transpiration rates (Larcher 1957) and properties of the leaf cuticle during dormancy. When stomata are closed in conditions of freeze-desiccation moisture loss is assumed to occur through cuticular transpiration (Peschl 1982). Baig and Tranquillini (1976) found the thickness of the needle cuticle and underlying cutinized cell wall layer of Norway spruce to be inversely proportional to cuticular transpiration during winter frost-drought stress. They reported decreasing cuticle thickness in spruce and pine needles with increasing altitude, and this correlated with increased transpiration. Hadley and Smith (1983, 1986) observed higher mortality of Picea engelmannii and Abies lasiocarpa needles due to wind exposure at high elevations in Wyoming.

Few investigations have been made on the seasonal properties of cuticles and rates of cuticular transpiration. Studies of this kind could be of considerable importance in validating the freeze-desiccation theory.

There is much controversy over the validity of the freeze-desiccation theory. Tranquillini (1982) reported that supporting experimental evidence is difficult to obtain, since observations of tissue desiccation may arise secondarily as a result of another injury. In a study of treeline species on Mt. Washington, New Hampshire, Marchand and Chabot (1978) did not find that the relative water content of entire balsam fir and black spruce foliage was excessively low during winter. In contrast, they found the relative water content of paper birch twigs to be very low, yet this species was frost hardy and survived winter without desiccation or freezing injury. Wardle (1981) noted that desiccation did not appear to cause "winter burn" injury, suggesting rather that direct freezing caused injury in winter. Tranquillini (1976, 1982) and Marchand and Chabot (1978) stated that direct needle exposure to sunlight is required to produce substantial leaf-to-air temperature gradients that promote moisture loss by cuticular transpiration. Vanderwaal and Holbo (1984), however, showed that leaf and air temperatures are closely coupled, which does not support the concept of Marchand and Chabot (1978). Observations from a current study in the
Peace River area do not support this notion. No overwinter damage to needle surfaces above the snow level has been observed. Needles covered by snow for long periods, however, appeared to lose some wax from non-stomatal areas, though wax plugs in stomata persisted.

It appears that white spruce in northeastern British Columbia may require additional stress, most likely low temperature, before foliar desiccation occurs. This conclusion is similar to that of Herrick and Friedland (1990) for red spruce. Recent studies of Peart et al. (1991) and Strimebeck et al. (1991) also fail to support the hypothesis of desiccation being the primary damaging agent for red spruce. The latter study concluded that, in red spruce, freezing injury and desiccation injury symptoms differ. Freezing injury resulted in reddening and needle abscission, whereas desiccation resulted in grey rather than red foliage and no needle abscission. This contradicts many descriptions of desiccation symptoms found in the literature. Regrettably, many studies of winter injury to conifers have concentrated only on needles.

Van Gardingen et al. (1991) showed that abrasive damage by wind of leaf surface wax could cause increased epidermal water vapour conductance, thereby potentially predisposing needles to desiccation. The authors' experiments were conducted with artificially created high-velocity winds in a wind tunnel. Marchand and Chabot (1978) concluded that ice abrasion was the primary cause of injury at high elevations, leading to needle desiccation and further damage. This theory dismisses high winds as a cause of increased desiccation injury, since high winds are thought to induce stomatal closure and therefore reduce transpiration rates. However, the question of stomatal opening in winter is also open to speculation as can be seen from the work of Strimebeck et al. 1991.

Kincaid and Lyons (1981) found no relationship between winter climatic conditions, the occurrence of foliage injury, and needle water potentials in red spruce. Neither did they find any obvious damage or inadequacy in the cuticle or cuticular plugs of the stomatal chambers in damaged specimens. Gibbons (1983) and Perkins et al. 1991 found no reduction in winter injury through the use of anti-transpirants on Scots pine and red spruce, respectively. Smit-Spinks et al. (1983) suggested that desiccation-like symptoms on winter-damaged Scots pine might follow some other form of tissue injury, where damaged cells had lost the ability to retain water. Venn (1979) concluded that frost-droughts in Norway spruce may be triggered by sudden changes of temperature between freezing and thawing. Other researchers have concluded similarly that freezing injury rather than water stress is the main cause of winter desiccation (Krarman 1986). Sakai and Otsuka (1970) compromise, suggesting that cold injury to plants is caused by several environmental factors, including rapid temperature changes and winter desiccation. A more recent study of red spruce in the northern Appalachians (Hadley et al. 1991) implicated desiccation, rapid temperature decrease, and deacclimation of foliage under intense solar heating as likely causes of injury. They also noted, however, that visible injury was preceded by desiccation.

Sakai (1970) concluded that winter desiccation injury affects conifers wintering under frozen soil and stem conditions for 2 or 3 months. Nevertheless, seedlings may ameliorate foliar moisture deficits during winter months by several means. During periods of elevated temperatures, snow cover melts and seedling stems thaw. Kincaid and Lyons (1981) suggested that under these conditions bulk water flow may occur, thereby replacing moisture lost previously through the cuticle. Cowling and Kedrowski (1980) observed that white spruce will absorb atmospheric water in the form of rain and melting snow directly through the leaves, thus increasing xylem water potentials. However, if seedling leaf conductance is abnormally high, desiccation stresses may reach critical levels before they can be corrected by these means. It is obvious from the above discussion that the debate over freezing versus desiccation injury is far from settled.

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3 Krasowski, M.J. and N.J. Livingston. Post-planting winter injury to seedlings on cold sites. For. Can and B.C. Min. For., Victoria, B.C. FRDA study (FR05).
4.3.2 Non-evaporative freeze-dehydration

Freeze-dehydration may not necessarily involve loss of water from the plant by evaporation (transpiration through stomata and cuticle). Removal of water from cells towards extracellular ice also produces a water stress effect even though the overall plant water content does not change (Levitt 1980).

According to Levitt (1972, 1980), freeze-dehydration results in two major types of secondary stress: increased solute concentration and mechanical effects. Other stresses are believed to involve molecular changes in the cell membrane.

Evidence has been presented to support the theory that dehydration can result in cell injury (see Levitt 1980 for review). A plant’s resistance to freezing and drought are closely correlated. Levitt (1980) concluded that extracellular freezing and resulting dehydration were the most important causes of freeze-killing in higher plants.

Explanations of the mechanisms of dehydration-induced injury implicate various denaturing* biochemical changes that may occur on removal of water from cells; however, much remains to be learned about this form of injury.

4.4 Interactions between Light and Low Temperature

Steffen and Palta (1987) suggested that photosynthesis is an important process governing plant responses to low temperatures. The capacity of a plant to use light is reduced at low temperatures, a phenomenon that may result in light-dependent injury (Öquist 1983; Powies 1984). Recent evidence shows that one of the photosystem II proteins is broken down and cannot be resynthesized at limiting temperatures (Guenther and Melis 1990), resulting in inhibition (photoinhibition) of photosynthesis. The capacity of the light-trapping system is unaffected, however, which leads to a metabolic imbalance in the chloroplast* (Gilles and Vidaver 1990).

While it is understood that incipient (sublethal) injury is repairable, the recovery process is energy demanding (Steffen and Palta 1987). The energy necessary for recovery must be supplied either by accumulated metabolites or directly from photosynthesis. High light levels may be destructive to the photosynthesis system, whereas low light intensity is thought to facilitate recovery (Greer et al. 1986). For example, reports suggest that low temperatures cause ultrastructural changes, particularly in chloroplast structure (disorganized thylakoid membranes), which impairs photosynthetic capacity (Martin and Öquist 1979). The sensitivity of chloroplasts to low temperatures and high light levels appears to depend on their stromal* concentration of Ca** (Charles and Halliwell 1980).

Steffen and Palta (1987) suggest that freeze-induced desiccation during prolonged freezing may alter photosynthesis by reducing the efficiency of stomata control. However, a full explanation of the relationship between freeze-induced changes in cellular ultrastructure and resulting energy balance problems is not yet available. Increased injury with prolonged stress is thought to result from a progressive destabilization at the molecular level involving:

- excess energy trapping with decreased utilization;
- high oxygen concentration; and
- formation of highly reactive forms of oxygen resulting in peroxidation* of membranes and protein denaturation (Halliwell and Guttenridge 1984).

In nature, light-dependent winter injury is demonstrated by the bleaching (loss of chlorophyll) of needles. Bleaching, considered to be a reversible injury, reportedly occurs prior to browning of the foliage, which indicates necrosis (Tao et al. 1987). These authors argued that photoinjury was the principal cause of tissue mortality in Korean pine seedlings during overwintering, and not desiccation or deacclimation as suggested by Zhu and Peng (1982) and Lu and Yang (1984), respectively.

Damage to conifer seedlings in Sweden has been blamed on clear skies and intense sunshine, which frequently occur in late winter and early spring (Christersson et al. 1987). Under such conditions, parts of young seedlings above the snow cover are injured by desiccation (Tranquillini 1982), photoinjury (Öquist 1983), or most likely a combination of both (Christersson et al. 1987).
4.5 Ice Pressure

Pressure created by the freeze-expansion of extracellular water may cause mechanical damage in the form of frost cracks in trees. There are conflicting views about the validity of this theory. This type of injury is limited to the cells within the crack, and does not cause injury to adjacent non-cracked tissues (Levitt 1980).

4.6 Air Expulsion and Smothering

Air expulsion is believed by some researchers to occur when ice expands in intracellular spaces. Evidence supporting this theory has not been convincing, however (Levitt 1980). Disruption of the gas exchange between tissue and air during prolonged ice encasement may cause injury even at relatively high temperatures (Levitt 1972, 1980). This could result in the accumulation of potentially toxic anaerobic respiration metabolites such as ethanol, CO₂, and lactic acid (Pomeroy et al. 1983). Ice encasement damage has been reported only for winter cereals, but this type of injury may also occur in forest tree seedlings.

5 A POSTMORTEM ANALYSIS IN NORTHEASTERN BRITISH COLUMBIA

Evident during this review of a winter seedling injury phenomenon in northeastern British Columbia has been the extreme complexity of plant adaptations for winter survival, and the large number of potentially damaging agents involved. In this section, the circumstantial evidence surrounding the phenomenon is more thoroughly assessed.

In view of the information presented so far, we believe the problem can be related to either one or both of the following factors:

- local environmental conditions in the Peace River area; and
- critical deficiencies in seedling characteristics.

5.1 Winter Climatic Patterns in Northeastern British Columbia

Since observations began at Stewart Lake in 1982, winter injuries to 1st-year spruce plantings have occurred each winter to 1991/92. At no time during this observation period has severe injury been observed on established, site-adapted spruces in the area. Levitt (1980) suggested that “test winters,” or those severe enough to damage plants of even intermediate hardiness, occur on average of once every 10 years. If some aspect of winter climate is responsible for the damage to young seedlings, it is unlikely to be one that is abnormal for the region. More probably it occurs annually and is not severe enough to injure established seedlings and natural regeneration. It should be noted, however, that naturally established seedlings, during their first growing season, do not usually reach the height of an average planted seedling and may spend the first winter predominantly under snow cover.

A typical winter air temperature record for the study area is illustrated in Figure 2, indicating the usual temperature regime experienced at Stewart Lake in 1986/87. Periods of intense cold (below -20°C) occurred during late October and early November. Snow rarely accumulates before November 1, leaving seedlings fully exposed to this early winter Arctic air mass. Snow cover gradually deepens during late November and early December, covering seedlings planted the previous spring.

Beginning in December, a widely fluctuating temperature regime begins, with daily maximum temperatures above freezing, followed by night temperatures to -10°C and lower. These periods of elevated, mid-winter temperatures occur when warm air masses blowing from the west temporarily displace frigid Arctic air masses during a weather phenomenon known as Chinook winds. During these Chinook events, high wind speeds, clear skies, and bright sunlight are common.
FIGURE 2. Typical Peace River area winter air temperatures (maximum and minimum) as measured at Stewart Lake at 1.5 m above the ground during the 1986/87 winter.

Throughout the period December to April, Chinook conditions may occur at any time. During 1985/86, two major warming periods occurred during mid-December and late February, when daily maximum temperatures rose above 10°C. Similar conditions occurred during 1986/87 in early and late February, and briefly again in late March, although maximum temperatures were somewhat lower than in the previous winter. Immediately following these warm periods, the temperature often drops sharply to -20°C or lower with the resurgence of Arctic air masses.

Snow accumulation on the plateau landform of the Peace River region is generally low compared to that on more mountainous terrain. Maximum snowpacks rarely exceed 100 cm. Snow cover is highly variable throughout the winter months and declines rapidly during periodic Chinook conditions. Observations in the winter of 1985/86 showed snowpack depths below 16 cm for most of the winter period. The snowpack was nearly eliminated during each of two Chinook events. While snow cover often recovered between warm periods, seedlings were usually exposed during the periods of elevated temperatures, high winds, bright days, and the sharp drop in temperature marking the end of the event. In contrast, during the 1991/92 winter, seedlings in the Peace River region were continuously under snow throughout the early and mid-winter, until the end of February when one powerful Chinook event eliminated snowpack completely.
5.1.1 Effect on young seedlings

The pattern of alternating mid-winter warm and intense cold periods characteristic of the Peace River area is suspected to cause injuries to young seedlings. The transient nature of winter snow cover may also be directly implicated, since newly planted seedlings are fully exposed to air temperature changes, drying winds, and intense light. All of these factors have been described in Section 4 as potentially injurious.

The rate of seedling tissue thawing and refreezing during Chinook events may subject seedlings to excessive stress and cell injury (Levitt 1972, 1980; Venn 1979). Also, the great repetition of diurnal and periodic freeze/thaw cycles is similar to the conditions causing injury reported by Christersson and Sandstedt (1978).

The influence of winter snow cover on the frequency of winter seedling injuries was observed at Stewart Lake. Snow accumulation in the south Peace River is influenced by prevailing winds, due to the relatively level terrain. Seedlings growing in the shelter of stumps, soil depressions, hillocks, and 3–5 m tall copses of hardwoods sustained either no injury or a very low incidence of injury. The "lee effect" of these microtopographic features resulted in deeper and more persistent snow accumulations over sheltered seedlings at all times during the winter than over unsheltered seedlings.

An experiment was designed to test the incidence of seedling winter injuries under two levels of snow cover at Stewart Lake. A concentric system of snow fencing was erected around planted seedlings to increase or maintain the normal accumulation of snow. The resulting snow catchment provided enhanced protection to 1st-year spruce seedlings compared with normal winter snowpacks for the site.

Observations made during the winter indicated that snow accumulation was faster, the depth of snow cover generally greater at any time during the winter, and the rate of snowmelt lower than within the snow catchment. The snow fence system did not maintain a homogeneous depth of snow or continuous snow cover during lengthy Chinook periods. However, the seedlings within the system were exposed to ambient air temperatures much less frequently than seedlings under normal conditions. The frequency of injuries was 40–60% lower where snow cover was enhanced.

The effectiveness of snow cover in moderating air temperature and therefore protecting overwintering container stock has also been reported (Gibbons 1983; Lindström 1986a, 1986b, 1987). During winter hardiness research in Ontario, a temporary loss of winter snow cover to field-stored container stock was one of the factors involved in serious losses of black spruce stock (Colombo 1982; Colombo and Glerum 1984).

Seedling exposure to drying winds and high levels of insolation during frozen soil conditions was discussed earlier. At Stewart Lake, L.J. Herring (unpublished) established trials to evaluate the importance of wind and sunlight as possible factors in seedling injuries. During late October, open-ended cardboard tubes 25 cm in diameter were placed over seedlings planted the previous spring. The tubes, tall enough to shade all seedling foliage, were staked in place.

The incidence of injuries to sheltered seedlings was nearly twice that of unsheltered seedlings by the following spring. Observations made during the winter indicated that the wind shelters interrupted the normal deposition of snow around the seedlings and sheltering tubes, resulting in less snow cover protection. It is also likely that the shelters altered the temperature distribution next to the enclosed seedlings. These conditions might have promoted a different kind of injury than that sustained by unsheltered seedlings. Thus, we could not derive more general conclusions from this experiment about the role of wind and sun in winter injury in the Peace River area.

Winter snow cover appears to be an important prerequisite for 1st-year seedling survival in the winter conditions common in the Peace River. Site treatments that maximize snow cover may encourage better survival during the all-important first winter. For instance, avoiding large clearings in otherwise featureless terrain may reduce the snow scouring effect of winter winds. Maintaining windbreaks of tall hardwoods or windrowed debris across the path of prevailing winter winds may encourage snow accumulation and retention.
Similarly, a moderate cover of hardwood saplings or non-crop brush may provide the necessary shelter. This strategy, however, requires further evaluation. Observations of a wide range of site preparation treatments at Stewart Lake suggest that brush cover does not guarantee accumulation or retention of snow cover and protection of seedlings during the winter. For instance, the presence of a dense cover (40,000 stems per hectare) of defoliated aspen shoots up to 1 m in height had little effect on either snow cover or the incidence of first-winter seedling injury. The density and size of brush cover protection required to affect snow cover dynamics may not lie within the range of acceptable competition for early plantation growth and management.

Clearly, newly planted seedlings are exposed to normal winter conditions in the Peace River. Sudden and drastic freeze/thaw cycles associated with cyclic air temperature change may be a major cause of injury, although the additional influence of desiccation or photoinjury cannot be completely ruled out. Also, the pertinence of root hardiness to the spruce winter injury problem is not yet clear. The presence of a snow cover likely moderates the temperature of the surface soil in the Peace River, providing freeze-sensitive roots with a margin of protection. However, it is not clear what effect Chinook warming and subsequent rapid cooling has on roots of 1st-year spruce seedlings. This phenomenon may be of more significance to root survival on certain exposed forms of planting microsite, such as inverted humus mounds or disc-trenched berms, than for undisturbed microsites. The subject warrants further study.

5.1.2 Effect on root injuries

Unlike winter injuries to shoots, root injuries are difficult to observe casually. Injury that does not kill but retards root growth is difficult to quantify (Lindström 1987). There is, however, a reported correlation between shoot injuries and reductions in root growth. Evidence of this relationship is provided by Colombo and Glerum (1984). They observed a 50% reduction in root growth capacity on black spruce seedlings that sustained injury to more than 25% of their foliage. They were unable, however, to determine whether shoot injury was a causal factor in reduced root capacity, or whether the roots themselves had suffered freezing injury.

Lindström (1986b) observed a corresponding decline in both root growth capacity and shoot growth with exposure to low root zone temperatures for 1+0 Norway spruce seedlings. In this case, no shoot injury symptoms were reported. In another study, however, Lindström (1986a) observed a delayed deterioration of foliage during the growing season following root zone exposures to -15°C. He attributed this gradual decline in shoot vigour to moisture stress resulting from root injury. Root injury results in water stress, which may suppress shoot growth (Steponkus et al. 1976; Smit-Spinks et al. 1985). Lindström (1986b) indicated that root injury occurs at relatively modest temperatures below the freezing point, suggesting that young roots are damaged more easily than older roots. Previously described injury symptoms, typically observed in the Peace River, suggest that winter injury to roots, if it occurs at all, is of lesser magnitude than that sustained by the aboveground seedling portion and does not appear to be lethal.

5.2 Seedling Characteristics

Since natural spruce regeneration and older planted seedlings appear to be less affected by winter injury than young planted seedlings, some characteristic of newly planted seedlings must predispose them to injury. Their temporary intolerance may be the result of morphological or phenological factors related to artificial regeneration.

5.2.1 Seedling phenology

The observed injuries to planted white spruce stock may be linked to freezer-storage and late spring planting practices. Freezer-storage of planting stock is known to retard bud burst (Ritchie and Roden 1985). Planting in the Peace River is undertaken in late spring, usually during the period of mid-May through June. This delay in budbreaking is an advantage in minimizing injury due to spring frosts. However, the combined effects of freezer-storage and late planting also appear to influence growth rhythm and phenology of outplanted spruce throughout the first growing season (Revel 1972; Revel et al. 1990).
A comparison of the 1st-year shoot growth phenology of white spruce planting stock and native, or site-adapted, trees is illustrated in Figure 3. It also appears from this figure that the method of phenological assessment may influence the conclusions. In the following discussion, the shoot growth phenology of adapted trees is compared with that in planted seedlings assessed by casual field inspections but without any strict methodology (observations at the Stewart Lake site), and with more organized, bi-weekly observations at Bear Mountain (similar altitude and latitude as Stewart Lake).

Adapted trees that have received the required chilling period during the previous fall and winter resume growth with the increased temperatures of early spring. The date at which active growth resumes varies by latitude and elevation for interior spruce species (Owens and Molder 1984). Studies of mature, high-elevation Engelmann spruce near Prince George (Harrison and Owens 1982) indicate that mitotic divisions in leaf primordia of terminal buds begin in mid-April, followed by resumption of mitotic activity in the apical meristem about 2 weeks later. Bud scale initiation begins next in terminal buds, followed by bud elongation, and finally bud burst and shoot elongation by early June (Owens and Molder 1984). Although no similar research has been undertaken in the south Peace River, the regional climate similarities suggest that similar dates of mitotic activity also occur there.

By comparison, freezer-stored planting stock is maintained at -2°C until early April. The stock remains in the dark at approximately +2°C to +5°C until removed for planting in mid- to late May. Temperature-induced active growth processes such as root activity and occasional bud flushes may begin before planting, in the +5°C storage. Changes dependent on elevated temperatures begin only after planting. For this reason, bud development and shoot elongation may be delayed for up to 4 weeks, unlike in site-adapted specimens. For example, observations at Stewart Lake indicate that bud flushing of seedlings planted on May 26 was delayed until June 16.

Shoot elongation is completed by the end of July in site-adapted spruce (Figure 3). The date appears to be similar for either low- or high-elevation varieties, and is assumed to apply to high-altitude provenances as well. Once shoot elongation ends, substantial morphological changes take place in the terminal and lateral buds of spruce. Bud meristems enlarge and leaf primordia are initiated until late September (high-elevation Engelmann spruce) or early October (low-elevation white spruce) (Harrison and Owens 1982; Owens and Molder 1984).

**FIGURE 3.** Shoot growth phenology of newly planted white spruce compared to native or site-adapted specimens in the Peace River. A) planted seedlings assessed in 1986 by casual observations at Stewart Lake; B) planted seedlings assessed bi-weekly during the 1988 growing season at Bear Mountain by arbitrary phenology classes (based on more than 50% of seedlings in the same class); C) site-adapted species, with assumed shoot growth phenology based on available literature.
By comparison, the period of active shoot elongation in 1st-year plantings is prolonged beyond that of site-adapted specimens. Shoot growth continues until at least mid-August under favourable growing conditions, although the endpoint of growth has not been thoroughly evaluated at Stewart Lake. Only where environmental conditions are severe, during a mid-summer drought, is shoot elongation prematurely curtailed. While site-adapted trees initiate the biochemical and morphological changes necessary for winter hardness development, 1st-year seedlings continue shoot elongation. The shift in phenology related to planting may forestall frost-hardening processes, which cannot take place during active vegetative growth. The winter hardness implications for seedlings 3–4 weeks out of phase with their environment may be substantial.

Observations at Bear Mountain differ from those obtained at Stewart Lake. Generally, the period of shoot elongation and bud development in late summer was shifted by only 2 weeks compared to that assumed for adapted trees. The difference in shoot growth phenology between planted and site-adapted spruces is likely influenced greatly by planting date. Consequently, altered shoot growth phenology may be creating a problem. Additional research in this area is needed.

Another possible source of problems may be that the majority of seedlings planted in the Peace River area are grown at southern, coastal nurseries. Schuch et al. (1989) found nursery environment to have a considerable influence on Douglas-fir seedling phenology when seedlings from the same seed sources were grown at different nurseries. Trees grown at coastal nurseries burst buds considerably earlier than did those grown at inland and northerly locations. Also, nurseries located at higher elevations, and sometimes those located at northern latitudes, produce seedlings that are more frost resistant than those from coastal nurseries. Although Schuch et al. (1989) admitted that the relationship between dormancy and frost hardness is still unclear, their observations are of importance.

Current studies are examining how the timing of bud burst in the spring following summer planting is related to the stocks’ nursery location. Because climatic conditions in the central and northern interior of British Columbia do not encourage early sowing for summer planting, the majority of summer planting stock is imported from southern nurseries. At the nursery, such stock is forced into budsetting and dormancy development early in the growing season so that it is ready for early summer planting. This may facilitate the tendency for early dormancy release during late winter Chinooks and in early spring. Hamilton (1973) reported that while extended periods of warm weather diminished frost hardness even in dormant plants, short exposures to warm conditions caused deacclimation only in non-dormant plants. Early dormancy release may then predispose seedlings to late winter damage, particularly following Chinook conditions during late winter months.

5.2.2 Cold hardness

The Peace River region experiences frequent Chinook events during the winter (December through March) with elevated temperatures and low snow cover. Site-adapted spruce seedlings and trees rarely appear damaged by these fluctuating temperatures. Natural acclimation apparently imparts sufficient winter-hardiness stability.

It is possible that inadequately hardened 1st-year seedlings deharden during exposure to one or more warm periods. Exposure to unseasonably warm weather for as short as 2 hours during the winter is known to decrease frost hardiness of conifers (Levitt 1980). Weiser (1986) reported that temperatures as low as +5°C can deharden plants by as much as 10° per day. Similar dehardening would put these seedlings at greater risk of cold injury during subsequent cold periods.

Ericksson et al. (1983) studied the effects of seedling storage (freezer-stored and outdoor-stored) and planting date (May through July) on the 1st-year frost hardiness and subsequent growth of 1+0 Norway spruce grown in paperpots. By mid-September, freezer-stored stock, particularly planted late, exhibited much poorer frost hardiness than seedlings stored outdoors before planting. Subsequent winter injury levels reflected these autumn frost hardiness differences. The authors also found that later planting dates had a negative effect on 2nd- and 3rd-year growth performance.
Freezer-storage and spring planting may affect the hardiness of container-grown white spruce more than that of bareroot stock. Tests of a variety of stock types at Stewart Lake indicated that bareroot stock sustained considerably less injury than did container stock types. At least two explanations for this trend are possible. In these studies, the 1st-year height growth for bareroot stock was substantially less than for containerized plants. Bareroot planting stress may have encouraged an earlier cessation of shoot growth, resulting in a greater period for dormancy induction and frost hardiness development. Alternatively, greenhouse culture under which the container stock types were grown may have resulted in different conditioning, possibly different growth rates, and different vegetative growth phenology after storage and outplanting, relative to the bareroot stock type raised under natural outdoor conditions.

Summer planting of non-stored stock appears to be the logical solution if freezer-storage and spring planting are the primary factors predisposing seedlings to winter injury. Indeed, it appears that summer-planted stock sustains somewhat less injury in winter than do the spring-planted seedlings. Summer-planted stock seems to be more susceptible to early spring bud damage (Figure A2, Appendix A), breaking buds easily the spring following planting, often during a period of frequent night frosts. As Lavender (pers. comm., 1992) noted, because of the chilling requirements of both spring- and summer-planted seedlings were long satisfied by the spring, the earlier bud breaking of the summer-planted stock could be akin to the effects of day length during the summer of planting. Summer planting is also often associated with considerable seedling mortality immediately after planting, due to summer droughts. These disadvantages of summer planting may outweigh the benefit of reducing winter injury.

The effects of nursery culture on white spruce seedling performance have been a continuing focus of nursery research in British Columbia. Higher quality standards for controlling shoot and root morphology, cold-storage and freezer-storage conditions, and root vigour have improved the performance of stock types in the field. Field tests of nursery treatments have, however, emphasized merely the performance parameters of active growth following outplanting.

The possibility of a nursery influence on other important seedling characters, such as winter frost hardiness, has received less study. Rowe (1964) suggested that the environmental influence of the previous year can precondition morphological and phenological attributes of tree seedlings during subsequent years. He warned of adaptation problems resulting from nursery preconditioning that could lead to the seedling being out of phase with its planting environment. He also suggested the possibility of controlling the nursery environment to "shape plants and seeds physiologically and morphologically, preadapting them to particular geographic localities as required."

Heide (1974) demonstrated the influence of the nursery environment on phenology and development of 1+0 Norway spruce seedlings in the 1st, 2nd, and 3rd years after planting. She found that both photoperiod and temperature during the period of bud differentiation (following cessation of shoot elongation) greatly affected the time of budbreak, shoot length, and date of shoot growth cessation during the following growing season. Seedlings that had developed buds in the nursery under long days and warm temperatures flushed 1–2 weeks later than those undergoing short-day treatments. Nursery after-effects were found to carry over to the 2nd and 3rd years following planting, although their magnitude declined. Heide (1974) also observed that growth of seedlings in the 1st year after planting was not just preformed growth. Instead, seedlings exhibited additional free growth. Shoot extension continued until later dates than in older site-adapted seedings.

While the capacity for (supplementary) free growth greatly increases the height growth performance of newly planted seedlings—a characteristic that most forest managers value—there are obvious risks involved. Pollard (1974), for instance, qualified the advantages of free growth with the need for sufficient time late in the summer for frost hardening and bud development. In a study of high-elevation trees, Tranquillini (1982) attributed inadequate cuticle development of species such as Norway spruce to short

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growing seasons and an inability of foliage to completely mature. Colombo (1984a) warned that spruce seedlings undergoing late cold hardening are unable to complete hardiness development when exposed to damaging fall frosts, making them susceptible to injury throughout the winter.

The goal of the Ontario “extended greenhouse culture” (Colombo 1984b) for preventing first-winter injuries to overwintering nursery stock is to extend the period of bud morphogenesis and cold hardening in the fall. This is done through artificially lengthening photoperiod in mid-summer, then switching to the natural short days of mid-August and September. The result is increased frost hardiness and winter injury reduction in outdoor-stored container stock. The effects of extended greenhouse culture on 1st-year outplanting performance and subsequent winter injury have not yet been fully evaluated.

5.2.3 Seedling morphology

It is possible that injury to shoot tips may be related to seedling height, as larger seedlings are more likely to have their uppermost portions above snow level for longer periods of time. Frequently observed localization of injuries between one-third and two-thirds of seedling height contradicts the assumption that shoot tips are more susceptible to damage just because they are more often above snow. Observed injury patterns suggest that those shoot portions most recently exposed after prolonged snow protection could be most susceptible to injury.

There is no proven relationship between stem thickness and susceptibility to stem injury, yet it may be speculated that thicker stems are somewhat less at risk, particularly to freeze-desiccation injury. Thicker stems may store more water in the xylem than do thinner ones and, with more favourable (lower) stem volume:leaf area ratio, thicker seedlings may withstand desiccation conditions longer when parts of the stem are thawed. An additional complication in evaluating the influence of morphology on a seedling’s susceptibility to winter injury is that different organs and tissues vary in their frost hardiness at different times (Alden 1971). Generally, short, sturdy seedlings have a better chance of surviving their first winter in the field than tall, thin seedlings. Some silviculturists believe that summer-planted seedlings suffer less winter injury to stems and needles than do spring-planted seedlings. The former, usually being shorter, may be better protected by snow and terrain irregularities. These beliefs, however, are based only on superficial observations and assumptions, and require verification.

The relationship between leaf surface morphology and susceptibility to desiccation has already been discussed. An inadequate cuticle on the needles of 1st-year spruce seedlings could result in excessive losses of water, leading to needle desiccation. However, current research indicates that spring- and summer-planted 1+0 and 2+0 seedlings of interior white spruce from northern provenances enter winter with well-developed cuticles, epistomatal chambers sealed with wax plugs, and very low cuticular conductance. However, when soil is frozen, lengthy snowless periods may result in gradual desiccation even at low cuticular conductances, as was observed in the central interior of British Columbia during the 1991/92 winter (M.J. Krasowski and N.J. Livingston, unpubl. data).

It is not known if and how the cuticular resistance may be lost during winter. These questions continue to be investigated. According to Cowling and Kedrowski (1980), white spruce is capable of stomatal opening and photosynthesis during warm mid-winter periods. They reported effective closure of stomata in response to moisture stress, which restricts subsequent moisture loss to cuticular evaporation. However, they found low xylem water potentials in white spruce during late winter in Alaska.

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5 Krasowski, M.J. and N.J. Livingston, FRDA Project FR05.
5.2.4 Seedling nutrition

It is not easy to separate the effects of seedling nutrition from other effects of nursery culture, since the nursery itself largely influences the mineral nutrition of the seedling during the first growing season in the field. The effects of nursery culture on seedling nutritional status in winter are likely more profound in summer-planted than in spring-planted seedlings. Summer-planted seedlings have a much smaller chance to be modified by the planting site, as they are planted after their shoot growth has been completed for the growing season.

Levitt (1980) includes the status of certain mineral nutrients among the many factors influencing winter hardness, particularly freezing tolerance. Nitrogen, phosphorus, potassium, and calcium—in overabundance and deficiency—have been implicated in inadequate winter hardening. Microelement deficiencies, including those of boron and copper, have also been implicated (Braekke 1979).

Aronsson (1980) evaluated the winter and growing season hardiness of Scots pine following fertilization studies of all of these previously mentioned macro- and microelements. He found that high levels of foliage nitrogen (exceeding 1.8% dry wt) reduced the level of frost hardiness measured during mid- to late winter. Growing season frost hardiness tests showed a similar trend in nitrogen content and frost tolerance, although greater variation was exhibited. High nitrogen levels were found to depress the levels of all other elements, particularly boron. Boron fertilization in a related laboratory study did not affect frost hardiness. In another study by Braekke (1979), boron fertilization of Norway spruce, Scots pine, and lodgepole pine on boron-poor peatland sites substantially reduced frost injury and shoot dieback.

Christersson (1973) found that different levels of potassium and magnesium fertilizer affected the water content and transpiration rate of Scots pine seedlings during the frost-hardening period, yet had little or no effect on frost hardiness. He also found that potassium is transported from the root to the shoot during the winter-hardening process, and that transpiration rates declined as foliar potassium levels increased. Both observations suggest that high foliar potassium levels may be important in protecting the seedling from winter moisture loss. Peschl (1982), however, found that nutrient-deficient Norway spruce were less able to control moisture loss only during the initial period of winter desiccation stress. Neither the general level of nutrition, nor any particular element affected cuticular transpiration rate.

Christersson (1975) tested the winter hardness of Norway spruce seedlings receiving a balanced fertilization regime against those receiving no fertilizer. Substantial differences in shoot growth were observed after 4 months, yet there were no differences in foliar nutrition levels and frost hardness development between fertilizer treatments.

A test of four levels of fertilization on white spruce 1+0 seedlings was conducted at Stewart Lake. Seedlings received either balanced slow-release, highly soluble NPK or highly soluble NP fertilizer materials at the time of spring planting. Although the uptake and resulting foliage nutrient concentrations for each treatment were not determined, no apparent benefit or detriment to winter injury susceptibility was observed.

Levitt (1980) noted many contradictory results of the effects of mineral nutrition on frost hardiness. More research is required before the relationship of seedling nutrition to frost hardiness is understood.
6 CONCLUSIONS AND RECOMMENDATIONS

Circumstantial evidence in northeastern British Columbia indicates that 1+0 white spruce plug stock that is overwintered in freezer-storage and planted during the spring is at high risk of first-winter injury. Associated factors include the aspect and exposure of the plantation (which affect the depth of snow cover) and a variable winter climatic pattern.

The type of injury sustained by seedlings has not been confirmed and the causes are currently being investigated. As well, the predisposing seedling factors have yet to be identified. Preliminary evidence suggests that both freeze-storage and late planting are involved, as they affect 1st-year phenology and frost hardiness development. There is also reason to suspect that nursery light and temperature regimes affect the development of seedling cold hardiness during the first growing season following planting.

Awareness of the significance of environmental injuries to forest plantations in British Columbia is increasing. In view of the paucity of knowledge on winter survival of outplanted white spruce, and the potential implications of poorly adapted growing stock in the Boreal and perhaps other biogeoclimatic zones, we recommend that the following areas be further investigated:

- **Diagnosis of tissue injuries**: To confirm the nature of tissue injury, dissection and analysis of injured tissues are required. Material for these analyses may be acquired from the field or artificially generated under controlled laboratory conditions. The effects of freezing soil temperatures on roots should be included in the analyses. The purpose of these tests should be to improve the accuracy of injury diagnosis, and so identify the primary damaging agent. This information should assist silviculturists in evaluating the extent of the problem in the field.

- **Identification of predisposing seedling factors**: A study of the post-planting seedling performance, phenology, morphology, and physiological status of a wide range of nursery stocks (or stock production treatments) may identify developmental problems, deficiencies, or maladaptations in seedlings, predisposing them to first-winter injuries. The phenological assumptions discussed earlier should also be confirmed or rejected through comparisons of recently planted seedlings with naturally regenerated or well-established planted ones.

- **Characterization of climatic patterns**: Further investigations of the macro/microclimate under which seedling injuries occur is required. A combination of field climate monitoring and environmental chamber tests should evaluate the range of air temperature, wind speed, and sun and snow conditions reportedly influencing seedling injury.

- **Conditioning seedlings for post-planting hardiness**: Nursery and field culture techniques must be evaluated and selected to maximize the adaptability of the seedling to its post-planting environment during both active growth and dormant seasons. The existing morphological basis for prescribing appropriate stock types may be inadequate for selecting the most appropriate seedling for sites with a risk of winter injury. More appropriate physiological parameters for evaluating seedling quality may be called for.
6.1 Current research

Two research projects devoted to the understanding and resolving of winter injury problems in the Peace River Region are currently under way. The recommendations above have been considered in the preparation of working plans for these projects. Detailed injury assessments are carried out on seedlings collected throughout winter from sites in the Peace River area. Seedlings of various seedlots, stock types, and planting dates are compared in these assessments. Climate monitoring is maintained on sites from which specimens are taken for injury evaluation and other assessments, so relationships between seedling condition and weather can be determined. Changes in relative water content of different seedling parts are monitored periodically between November and May to determine the relative water content of these parts. Measurements of leaf chlorophyll content are taken at the same times to evaluate the extent of bleaching due to possible light injury. The same treatments (stock types, planting dates, open or sheltered sites, etc.) as those in injury evaluations are compared in these measurements. Winter survival on some operationally planted sites (differing by opening size, elevation, aspect, site preparation, etc.) is also monitored.

Another part of these studies deals with changes in frost hardiness induced by winter warming events. Seedlings of various types are exposed to artificially induced thawing at different times in winter. A portable greenhouse with electronically controlled temperature is used in these experiments. After an exposure to warm temperatures—similar to those occurring during Chinooks—frost hardiness of the exposed seedlings is compared to that of unexposed controls. Thawed seedlings are then exposed to ambient winter temperatures and, after some time, are evaluated for injuries and compared to the controls.

Studies of seedling water relations are also carried out on seedlings of several stock types, seedlots, nurseries of origin, planting dates, and levels of exposure to wind and sun (sheltered or not). Changes in a number of water relation parameters, particularly osmotic potential, are carefully studied throughout the growing and dormant seasons. Special consideration has been given to seasonal changes in cuticular conductance, as it is indicative of seedling ability to withhold water under transpiration-promoting conditions. Leaf surface features related to cuticular conductance, such as the amount and configuration of leaf surface wax, are also studied.

The studies described above are still in progress, but some observations indicate that sheltering the southwestern side of outplanted seedlings may considerably reduce winter injury by reducing light exposure and prolonging the presence of snowpack. The sheltering method must be operationally applicable. Strip-cutting through aspen stands and planting spruce in the cleared strips may be worth studying. The widths and directions of the strips could be chosen such that remaining aspen would effectively serve as a shelter to the planted seedlings.

It is hoped that studies currently under way will identify the major causes of winter injury that occur so frequently in the geographic area described in this text, and will lead to strategies to alleviate this problem.
7 LITERATURE CITED


APPENDIX A: Examples of winter injury

FIGURE A1. The range of winter injury to planted spruce seedlings. A) Severely damaged seedling planted in the spring of the previous year. Injury affected most of seedling’s foliage, but more browning occurred at the southwest side (the facing side). Some green foliage is present at the shoot tips and at the basal portion of the seedling. Photographed in March 1991. B) Seedling with extensive foliage injury and dead lateral shoots in the mid-portion of the plant. However, green, live foliage is present at the seedling base. The top one-third appears to be less damaged, some needles are green, and most buds have flushed. Photographed in mid-May, 1991. C) The whole aboveground portion of this seedling was killed, except a few branches at the seedling base. Photographed in mid-May 1991.

FIGURE A2. Freezing injury to interior spruce seedling planted the previous summer. A) Summer-planted seedling killed during winter. B) Spring frost damage to interior spruce seedling showing damaged young shoots that were injured shortly after bud flushing.
APPENDIX B: Glossary

chloroplast  cell organelle (a plastid) that contains chlorophyll; the site of photosynthesis and starch formation.
cryobiology  a branch of science dealing with the effects of very low temperatures on biological systems.
denaturate  deprive of natural qualities.
endocytic vesiculation  pinching off of membrane material to form vesicles; the vesicles are pinched off into the cell cytoplasm rather than to the outside.
endogenous  caused by factors located deep within the organism or system.
epistomatal chambers  depressed opening of a stoma serving in gaseous exchange between a leaf and the atmosphere.
extracellular  pertaining to the space outside a cell.
free growth  growth by simultaneous initiation and elongation of new leaves and stem portions (stem units) rather than by elongation of overwintering preformed shoot. Free growth occurs typically in many conifers between germination and the first budset; in later years it is usually replaced completely by preformed growth.
hydrop Hillic  having strong affinity for water.
intercellular spaces  spaces between cells.
intracellular  within a cell.
lysis  breaking down; disintegration.
nucleating  causing formation of clusters.
osmotic potential  the ability of a solution to produce pressure by or associated with osmosis (a movement of a solvent through a semipermeable membrane into a solution of a higher solute concentration), and dependent on molar concentration and absolute temperature.
parenchyma  a common tissue of higher plants characterized by cells with thin walls.
peroxidation  chemical reaction with peroxides, i.e., oxides that contain a high proportion of oxygen and are highly reactive (e.g., hydrogen peroxide). plasma membrane  a sack-like semipermeable membrane; the outermost layer of the protoplast.
plasmolysis  shrinking of the cytoplasm away from the wall.
preformed growth  growth exclusively by extension of a preformed shoot that is enclosed within a bud during winter.
protoplast  in plant cells, a cell minus cell walls; protoplast consists of the plasma membrane, cytoplasm, and nucleus.
quiescence  inactivity.
stroma  chloroplast matrix in which the membranes (lamellae) are imbedded.
supercooling  cooling below freezing point without solidification.
thermotropic  change in orientation caused by temperature gradient.
thylacoid membranes  membranes of a chloroplast that are the site of photochemical reactions of photosynthesis.
ultrastructure  the invisible ultimate physiochemical organization of protoplasm.